A COMPARATIVE EXPERIMENTAL & CLINICAL STUDY OF N-B-PHENYLETHYL-ANTHRANILIC ACID (TROMARIL) & SOME NEW NON-STEROIDAL ANTI-INFLAMMATORY AGENTS

THESIS FOR
DOCTOR OF MEDICINE
(PHARMACOLOGY)

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SADHNA

C E R T I F I C A T E

Certified that the work entitled "A COMPARATIVE EXPERIMENTAL AND CLINICAL STUDY OF N-β-PHENYL-ETHYL ANTHRANILIC ACID (TROMARIL AND SOME NEW NON-STEROIDAL ANTI-INFLAMMATORY AGENTS" has been carried out by DR. SADHNA herself in this department.

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C E R T I F I C A T E

This is to certify that the work of Dr. SADHNA, "A COMPARATIVE EXPERIMENTAL AND CLINICAL STUDY OF N-B-PHENYLETHYL ANTHRANILIC ACID (TROMARIL) AND SOME NEW NON-STEROIDAL ANTI-INFLAMMATORY AGENTS", which is being presented by her as a thesis for the award of M.D.(Pharmacology), has been carried out by the candidate under our guidance and supervision. The techniques employed in this work were actually undertaken by the candidate herself and the observations were periodically checked by us.

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Sadhna

CONTENTS

INTRODUCTION

Chronic arthritis is one of those crippling diseases which shows no mercy to age and may creep into life of an individual at any time from childhood to old age. Although, rehumatism is one of the oldest known disease and affects a large percentage of population of the world, yet a satisfactory treatment is not available. Various pharmacological agents have been advocated for its treatment. Salicylates indomethacin, ibuprofen, phenylbutazone, naproxen and glucocorticoids are very commonly used for the treatment of arthritis. Gold salts, Dpenicillamine (Jaffe, 1965) and immunopromoters like B.C.G. vaccine (Rewald, 1974), levamisole (Huskisson et al, 1978) and tolmetin (Carson et al., 1971), are the recent additions to the pharmacological armamentarium against rheumatoid arthritis. During past quarter century, significant advances have been made in our understanding and management of the rheumatic group of the diseases.

Despite intensive research we still do not know the cause nor do we have a cure for one of the most serious problems - Rheumatoid arthritis "The enemy one knows is less dangerous than the enemy one does not" applied well to this great crippler.

Inspite of availability of a large number of drugs, the disease remains incurable. This failure can be attributed to the ignorance of the aetiology of disease and a relative ineffectiveness and troublesome side effects of

drugs on prolonged use. Search is going on to explore new and novel chemical compounds, safer and better tolerated, than existing therapeutic agents.

Based on the concept of structure activity relationship, a number of new organic molecules were synthesised at Regional Research Laboratory, Hyderabad. These were subjected to pharmacological screening. Several of these compounds possessed interesting anti-inflammatory activity.

One of these compounds - tromaril (N-3-Phenylethyl anthranilic acid) was studied for its pharmacological and toxicological profile. It has anti-inflammatory analgesic and antipyretic properties in various animal experimental models (Sisodia et al, 1980). Tromaril has shown a good degree of safety in animal toxicity experiments (Sisodia et al, 1980) including teratogenic (Sisodia et al, 1980) and mutagenic studies (Polasa and Shobha, 1980). In rats, the drug has very little ulcerogenic activity when compared with phenylbutazone (Sisodia et al, 1980). These observations have been corroborated in clinical situations (Rao et al, 1980; Swamy et al, 1980; Mathur et al, 1980).

This study was undertaken with following aims in view :

1. Confirmation and comparison of analgesic, antipyretic, anti-inflammatory and ulcerogenic activity of tromaril with aspirin, indomethacin, brufen and tolmetin in animal experimental models.

2. Comparative study of tromaril with aspirin in diagnosed cases of different types of arthritis.
3. Comparative toxicity studies of tromaril with aspirin, brufen, tolmetin and indomethacin.
4. Study of incidence of side effects in patients under treatment.

REVIEW OF LITERATURE

Arthritis, the most distressing and disabling syndrome encountered in medical practice, has been aptly termed as "Great crippler" and the "King of human miseries".

Inflammation is a complex vascular, lymphatic and local tissue reaction elicited in higher animals by the presence of viable or non-viable irritants (Menkin, 1956). The cellular events in the injured tissue include fenestration of the micro-vascular, leakage of the elements of the blood into the interstitial spaces and migration of leucocytes into the inflamed tissue. On macroscopic level, this is characterised by erythema, edema, tenderness and pain (Goodman and Gilman, 1980). The term "Rheumatoid arthritis" was coined by Garrod in 1859 for an inflammatory affection of the joints. Rheumatoid arthritis, a chronic polyarthritis affecting the peripheral joints with exacerbations and remissions, probably involves the combination of an antigen (gamma globulin) with an antibody (rheumatoid factor) and a complement.

Pathophysiology of inflammation :

Inflammatory reaction has essentially three features - (1) dilatation of blood vessels and increased vascular permeability leading to erythema and oedema at the site of noxious stimulus (Transudative phase), (2) cellular infiltration (exudative phase) and (3) finally tissue repair (proliferative phase) (Bhargava and Gupta, 1977).

The classical signs of inflammation include redness, swelling, heat and pain (Conheim, 1882).

Inflammation may very from the acute transient and highly localised response to simple mechanical injury or the complex persistent response involving the whole organism. It initiates a series of biochemical, immunological and cellular events which may follow the initial responses and ending with physical repair and restoration of the function of the injured tissue (Naik and Sheth, 1976). This complex response involves the liberation of chemical mediators like histamine, 5-hydroxytyptamine, S.R.S. - A, Kinins and prostaglandins. Every pathological condition is associated with tissue injury hence inflammation may be the commonest change observed, though not necessarily in acute form. Remarkable resemblence has been observed in response to a wide variety of stimuli. The nature and consequences of the reaction may vary depending on the type of a stimulus and the defence capacity of the host. The inflammation can be grouped under various heads depending on the response of the tissue to injury.

a. Acute inflammation - When the tissue damage is triggered by mechanical trauma, thermal injury, chemical burn or acute allergy, it represents an early reaction followed by repair (Naik and Sheth, 1976).

b. Chronic inflammation - When an irritant of low grade intensity acts upon the tissues it results in chronic inflammation. It is characterised microscopically by the

presence of lymphocytes, plasma cells and macrophages. Chronic inflammation is usually a sequel to acute inflammation or may be chronic from the beginning and the resulting fibrosis is more marked than acute inflammation (Boyd, 1974).

Chemical mediators of inflammation :

a. Histamine - The concept of endogenous histamine was first postulated by Lewis (1927). Subsequently, it was reported that histamine itself is released by injury (Spector, 1956; Bhatt and Sanyal, 1963; Bhalla et al., 1970). However, Bhargava et al (1976) observed that increased capillary permeability induced by histamine is mainly due to activation of H_1 receptors as it was completely blocked by mephyramine. However, H_2 receptor blocker (Burimamide) failed to inhibit histamine - induced increased capillary permeability. Therefore, H_1 - receptor blockers may be regarded as having anti-inflammatory activity.

b. 5-hydroxytryptamine (5-HT) - Rowley and Benditt (1956) observed oedema of rat paw on injection of serotonin (5-HT). Serotonin as well as histamine (Parratt and West, 1957; Bhatt and Sanyal, 1963) are liberated during early phase of inflammation and like histamine it might also be responsible for vascular reaction and the oedema formation after injury (Wilhelm, 1962). The permeability effects of 5-HT might be attributed to a direct effect on vascular epithelium or to the release of histamine (Spector, 1956).

c. Prostaglandins - Prostaglandins are considered to be chemical mediators of inflammatory reaction. Prostaglandins of E type are reported to suppress acute and chronic inflammation in normal and adrenalectomised rats (Zurier et al, 1973) and also are held responsible for initiation and maintenance of inflammatory process. Crunkhorn and Willis (1969, 1971 a, 1971 b) observed vasodilatation, increased capillary permeability and migration of leucocytes by prostaglandins in rat and man. Synergistic effects on capillary permeability are seen between prostaglandins and bradykinin, histamine or 5-HT in experimental animals (Sekemeier et al, 1974). Willis (1969) and Di Rosen et al, (1971) showed the presence of prostaglandins in the inflammatory exudate and further reported that carragennin induced - oedema in rats is mediated by histamine and 5-HT during first hour while increased vascular permeability is maintained by Kinin release upto 2½ hours. However, between 2½ to 6 hours the mediator appears to be prostaglandin, the release of which is closely associated with migration of leucocytes. When the acute phase of inflammation is over, prostaglandins have been reported to promote granuloma formation, mediate collagen metabolism (Raisz and Kooleman Beynen, 1974) and activate mucopolysaccharide synthesis (Peters et al, 1974).

d. Polypeptide - The term "Bradykinin" was coined by Rocha-e-Silva et al, (1949) and was reported to cause vasodilatation, increased capillary permeability and smooth muscle stimulation. It possesses all characteristics

of "chemical mediator of inflammation" as increased capillary permeability (Haldstock et al, 1957), pain (Armstong, et al, 1957) and migration of leucocytes (Lewis, 1962).

e. Proteases - Two types of proteases - Kallikrein (Spector and Willoughby, 1968) and plasmin (Macfarlane and Pilling, 1948) have been identified during inflammatory process. Kallikrein has been reported to release kinins from their inactive precursor showing increased capillary permeability (Spector and Willoughby, 1968). Proteolytic enzyme (fibrinolysin or plasmin) might be an important link in inflammatory process (Macfarlane and Pilling, 1948).

f. Other factors - Factors like leucotoxin, leucocytosis promoting factors and lymphnode permeability factor (LNPF) have been shown to be responsible for causation of increased capillary permeability during inflammation (Hurley and Spector, 1961; Willoughby and Spector, 1964). Further, uncoupling of oxidative phosphorylation results in reduced biosynthesis of ATP which causes inhibition of mucopolysaccharide synthesis and inflammatory and tissue growth (Adams and Cobb, 1958). Steroidal and non-steroidal anti-inflammatory agents have been reported to uncouple oxidative phosphorylation (Whitehouse and Haslam, 1962).

Probable mode of action of nonsteroidal anti-inflammatory drugs (NSAID) :

Inhibition of prostaglandin synthesis is the most

widely accepted mechanism of action proposed for NSAID (Vane et al, 1971). Ferriera and Vane (1974) demonstrated inhibition of prostaglandin synthesis by anti-inflammatory agents. The current opinion holds that during inflammatory hyperalgesia, fever and platelet aggregation, arachidonic acid is released from the phospholipid fraction of the cell membrane by the action of phospholipase A_2. Corticosteroids have been reported to inhibit the prostaglandin synthesis apparently by inhibiting the substrate or inhibiting the action of phospholipase (Flower, 1974), thus resulting in complete inhibition of all the proinflammatory mediators (Figure 1). NSAID are shown to decrease synthesis of cylic endoperoxide (PGG_2 and PGH_2) by inhibiting the enzyme cyclooxygenase. Kuehl and associates (1971) reported that PGG_2 plays significant role in inflammatory process and further showed that free radical liberated upon conversion of PGG_2 to PGH_2 may regulate the formation of pro-inflammatory mediators. PGH_2 is acted upon by isomerase and reductase contained within the prostaglandin synthetase complex producing PGE_2 and PGF_2 alpha. NSAID by inhibition of cyclooxygenase reduce the levels of PGI_2, PGE_2, PGF_2 alpha and TXA_2. Arrigony - Martelli (1977) and Shen and Winter (1977) have reported the other possible mechanisms of action of NSAID as follows :

1. Inhibition of chemotaxis of cells involved in inflammation.

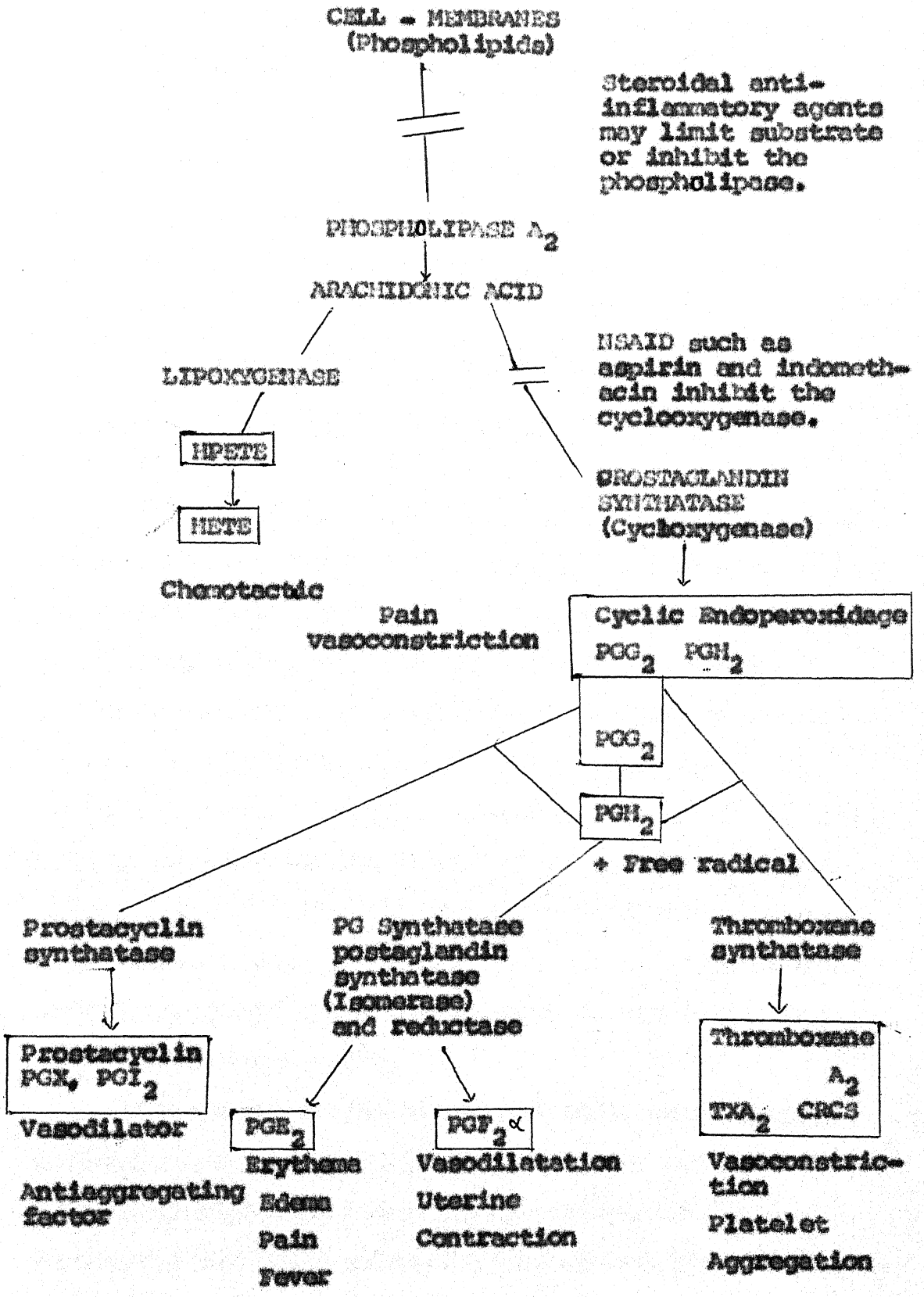


Figure - 1 : Generation of potential mediators of inflammation by enzymatic conversion of arachidonic acid.

2. Inhibition of lysosomal membrane inhibilization, antagonistic effects on mediators other then prostaglandins e.g. Histamine, 5-HT, SRS-A, Kinins, lymph node permeability factor (LNPF), peptidases, complements and others.
3. Inhibition of the biosynthesis of mucopolysaccharides.
4. Uncoupling of oxidative phosphorylation.

History of anti-inflammatory drugs and discovery of Tremacil :

Rheumatoid arthritis being one of the most painful and crippling disease entity, affects a major part of the population of the world, yet a satisfactory treatment is not in sight. The study of inflammation and anti-inflammatory drugs occupied an important place in therapeutic armamentarium and the anti-rheumatic activity of cortisone was reported by Hench et al, (1949) and for this outstanding contribution, he was awarded the Nobel prize. The highly potent corticosteroids, are not devoid of adverse effects, require therapy for prolonged period. Therefore, it necessisted the search for non-steroidal anti-inflammatory agents.

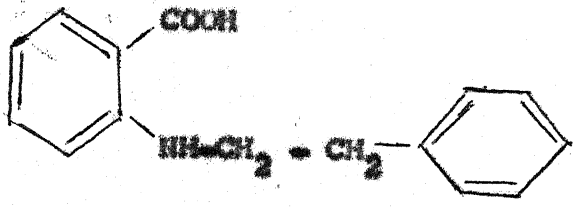
Various drugs like glucocorticoids, non-steroidal anti-inflammatory agents like salicylates, indole acetic acid derivative like indomethacin, anthranilic acid derivative like mefenamic acid, arylalkanoic acid derivative like ibuprofen, naproxen, ketoprofen, anti-malarials

like chloroquine, gold salts, chelating agents like D-penicillamine (Jaffe, 1965) and immunopromoters like B.C.G. vaccine (Rewald, 1974) and Levamisole (Huskisson et al, 1978) constitute the main study of the treatment of this disease. Moreover, inspite of the availability of large number of drugs, this disease still remains incurable. All the anti-inflammatory drugs produce undesirable effects like epigastric pain, gastrointestinal haemorrhage, and gastrointestinal ulceration, besides they very frequently interact with other drugs then affecting treatment. Therefore, search for safer non-steroidal anti-inflammatory agents is still on. In the intensive search of a new but safer non-steroidal anti-inflammatory agent synthetic analogues of authranilic acid has been synthesized and subjected to pharmacological screening.

Fenamates belonging to anthranilic acid derivatives evoked considerable interest in view of their anti-inflammatory, analgesic and antipyretic activities. Amongst these, mefenamic acid, flufenamic acid and meclofenamic acid are available as an anti-inflammatory and analgesic-antipyretic agents. However, they are not free from undesirable effects shown by the other anti-inflammatory agents.

In view of the foregoing, modified analogues of these compounds were synthesized to obtain more potent drugs with minimal side effects. It resulted in the synthesis of new compound, N-β-phenylethyl anathranilic

acid, code named Ri-8 (Tromaril), possessing potent anti-inflammatory, analgesic and antipyretic activity (Sisodia et al, 1966).



Chemical structure of Tromaril :

It is a light buff-coloured solid, having a melting point of 117°C - 118°C, insoluble in water and soluble in common organic solvents. It has a pka value of 5.25 ± 0.025. Its IR spectrum shows co-absorption at 1670 cm^{-1} cms and NH at 3360 cm^{-1}. It is prepared by condensing 2-chlorobenzoic acid with p-phenylethylamine.

Pharmacological actions of tromaril in experimental animals :

Sisodia et al, (1980) reported dose-related anti-inflammatory activity of tromaril and found it to be equipotent with phenylbutazone in inhibiting carrageenin induced paw oedema, resin pellet granuloma, adjuvant arthritis and formalin peritonitis in rats. However, it was half as potent as oxyphenbutazone in delaying onset of ultraviolet erythema in guinea pigs and twice as potent as phenylbutazone in inhibiting mouse ear oedema. The inhibitory effects of tromaril on wound healing in rat and on granulation tissue formation on chorioallantoic membrane of chick embryo paralleled with phenylbutazone

(Sisodia et al, 1990). Adrenalectomy failed to influence the inhibitory effect of tromatil.

Since the anti-inflammatory agents possess analgesic activity, it showed analgesic activity in rats similar to phenylbutazone (Sisodia et al., 1990) and was subjected to further studies involving analgesic activity. However, it was found to possess greater antinociceptive activity than aspirin and mefenamic acid as assessed by Haffner tail clip, Eddy's hot plate and radiant heat method (Sisodia et al., 1990).

Anti-inflammatory agents are found to possess antipyretic activity. Therefore, tromaril was tested to assess this property (Sisodia et al, 1990). Tromaril has same antipyretic activity as oxyphenbutazone on yeast-induced pyrexia in rats (Sisodia et al., 1990). However, in contrast with phenylbutazone, tromaril failed to show any ulcerogenic activity upto the dose of 400 mg/kg in rats (Sisodia et al., 1990). Tromaril was devoid of any enzyme induction activity in rats as compared to phenylbutazone. It inhibited degranulation of mast cells in rat following aseptic injury (Sisodia et al., 1990).

No significant effect was observed on cardiovascular system, central nervous system, respiratory system, and smooth muscle in experimental animals (Sisodia et al, 1990). Tromaril possesses a wide margin of safety as evidenced by I.P. LD_{50} values in mice (760 mg/kg IP rats), and oral LD_{50} value in mice, rats and dogs (> 2000 mg/kg).

Tromaril has been reported to inhibit prostaglandin synthetase responsible for its anti-inflammatory, antiplatelet aggregation activity like indomethacin (Kashiramgar et al., 1980). However, indomethacin decreases but tromaril increases urinary volume and electrolyte excretion (Kashiramgar et al., 1980). Further it has been postulated that indomethacin is one of the most potent inhibitors of prostaglandin synthesis (Goodman and Girlman, 1980).

Tromaril pretreatment inhibited significantly liver succinic dehydrogenase, glutamic oxaloacetic transaminase glutamic pyruvic transaminase and acid phosphatase content of oedematous or granulomatous tissue during carrageenin edema and cotton pellet granuloma (Naik and Sheth, 1980). It also inhibited the alteration in the connective tissue components like hydroxyproline, hexosamine and sialic acid during carrageenin oedema and cotton pellet granuloma (Naik and Sheth, 1980). It is postulated that tromaril might inhibit the inflammatory process by inhibiting many biochemical processes at cellular level (Naik and Seth, 1980).

Valsma et al., (1980) reported that tromaril failed to cause haemolysis of G6-PD deficient red cells in vitro.

Pharmacokinetics of Tromaril :

Pharmacokinetic studies have been done in dogs and rats by Sisodia et al. (1980). Tromaril was found to be rapidly and well absorbed after oral administration and 50% of total amount of drug was absorbed within half an

hour (Sisodia et al., 1980). Thirty minutes after oral administration the peak plasma level of the drug was 3.2666 mg/ml in rat and 8 mg/ml in dog. Plasma half life was reported to be 18 minutes in rats and 21 minutes in dogs after intravenous administration (Sisodia et al., 1980).

Highest concentration of tromarit was found in liver. It crosses the blood brain barrier and significant portion is bound to plasma proteins (Sisodia et al., 1980). Tromaril was found to be excreted in urine for over 72 hours after a single oral dose, which is attributed to slow release of the drug from binding sites in tissues. The faecal excretion of the drug was continued for over a period of 72 hours after single oral dose. Therefore, the drug undergoes enterohepatic circulation as evidenced by the presence of drug in bile (Sisodia et al., 1980).

Bio-availability studies of Tromaril :

Rao et al. (1980) showed that peak serum concentration is achieved in about two hours after oral administration which was found to be dose-related. The blood level declined in 4 hours (Rao et al., 1980). Presence of food has been reported to increase the absorption of tromaril (Kashiramgar et al., 1980).

The acute, subacute and chronic toxicity of tromaril (RH-8) has been studied in mice, rats and dogs. The intraperitoneal LD50 value in mice was 760 mg/kg. However, oral LD50 value in mice, rats and dogs was found to be

greater than 2000 mg/kg. The subacute and chronic toxicity study of tromaril showed that it was well tolerated by rats and dogs. Further, no significant pathological changes, morphological or functional, was observed at the oral low dose level. In long term study, no significant haematological, chemical or pathological changes were observed at the low dosage level on oral administration (Sisodia et al., 1980).

Tetratogenic effects of tromaril were investigated in rats and rabbits. On oral administration of tromaril to pregnant rats (20-640 mg/kg/day) and rabbits (20-200 mg/kg/day) during the organogenesis period, no teratogenic effect was reported on foetuses. Moreover, tromaril was devoid of any effect on post-natal development of their pups (Sisodia et al., 1980). In the mutagenic studies, the incidence of micronuclei and P/N ratio in bone marrow erythocytes in tromaril and brufen-treated mice was reported to be similar to the untreated controls indicating lack of induction of any genetic damage by these drugs (Shobha Devi and Pelaza, 1980). However, phenylbutazone and indomethacin showed a significant increase in the frequency of micronuclei in the polychromatic erythocytes and altered the P/N ratio.

Clinical studies of tromaril :

Gastro-intestinal haemorrhage has been reported after the administration of corticosteroids (Kowalewski 1959), salicylates (Marchetti et al., 1974) mefenamic acid

(Wolfe et al, 1976), indole and phenylpropionic acid derivatives (Bhargava, 1973). Significant increase in blood loss was reported after administration of aspirin, flurbiprofen and tromaril (Dahanukar et al, 1980) but only the difference between tromaril and flurbiprofen group is significant. Tromaril has been found to possess significant antithrombotic activity (Manikeri et al, 1980). Tromaril and flurbiprofen were reported to inhibit platelet aggregation without affecting coagulation tests. On the contrary, aspirin inhibits platelet aggregation in some tests of coagulation (Manikeri et al, 1980; Gupta et al, 1980). Tromaril has been reported to be effective and safe in arthritis, arthralgia, osteoarthritis and gout as anti-inflammatory drug in view of their good gastric tolerance and devoid of side effects (Swamy et al, 1980; Mathur et al, 1980; Sattur et al, 1980; Rao et al, 1980). Rao (1980) observed significant anti-inflammatory analgesic effect in rheumatoid arthritis. No untoward effect was observed on haemopoetic, renal or hepatic systems after the use of tromaril (Sisodia et al, 1980). Rao et al, (1980) and Lokhani et al, (1980) reported tromaril and oxyphenbutazone to be equally effective.

Tromaril showed antipyretic activity equal to that obtainable with aspirin (Gupta et al, 1980). Raste et al, (1980) failed to detect the presence of tromaril in breast milk of lactating mothers.

In contrast with indomethacin, tramadol was reported to increase the urinary volume, sodium, potassium and protein output significantly in patients with nephrotic syndrome (Kashirsagar et al, 1980).

Properties of other anti-inflammatory drugs :

After the discovery of aspirin in 1899 by Dresser, various anti-inflammatory drugs were introduced such as aminophenols like phenylbutazone, indole acetic acid derivatives like indomethacin, anthranilic acid derivatives like mefenamic acid and arylalkanoic acid derivatives like ibuprofen, naproxen, ketoprofen. However, amongst these drugs aspirin remains the drug of choice for rheumatic and arthritis conditions (Goodman and Gilman, 1980). Indomethacin, synthesised by Shen et al.(1963), was considered to be more effective than any other anti-inflammatory drug for the long term management of chronic arthritis with minimal toxicity to the patient (Norcross, 1965). In studies conducted by Winter et al,(1963), higher efficacy of indomethacin over aspirin for its anti-inflammatory action was established as it was found to be 25 times as potent as aspirin in hind paw oedema and cotton pellet tests. Moreover, indomethacin was reported to be more potent anti-pyretic agent by these workers. Indomethacin has been reported to be better than placebo in rheumatoid arthritis in clinical studies (Deodhar et al, 1973). Aspirin and ibuprofen have been reported to be more effective than placebo and equally effective in analgesic activity

(Gimsiracusa et al, 1975). Objective assessments of joint measurement were found to be similar for ibuprofen and indomethacin (de Blecourt, 1975).

Tolmetin has been reported to possess a marked anti-inflammatory activity as it inhibited carrageenin-induced paw oedema (Carson et al, 1971; Wong, 1975). It was more active than aspirin but less active than indomethacin (Amouters et al, 1975; Wong, 1975). Most of the anti-inflammatory drugs possess analgesic activity. However, unlike established analgesic action of aspirin (Winder, 1959; Paalzow, 1969; Dubas and Parker, 1971), the analgesic activity of indomethacin, distinct from its anti-inflammatory effect remains uncertain. Collier et al. (1969) reported it to be an effective analgesic in abdominal constriction response. Tolmetin failed to respond in Haffner tail clip assay, however, it significantly inhibited acetic acid-induced writhing (Sangal, 1982). Salicylates increased cardiac output and right ventricular and systemic pressures (Tehney and Miller, 1955). Aspirin and indomethacin, which inhibit prostaglandin synthesis, enhance the coronary dilatation induced by increased cardiac activity, hence might be useful in preventing coronary insufficiency in conditions of cardiac stress (Talesnik and Sunahara, 1973). Aspirin is most vulnerable in producing initial respiratory stimulation followed by depression, which leads to respiratory alkiosis followed by metabolic acidosis (Rosenstein and Dorison, 1963).

While observing the central nervous system effects of anti-inflammatory agents, headache drowsiness and depression, were observed with indomethacin (Healey, 1967) and Naproxen (Katona, 1973) whereas salicylates are likely to cause significant neurological effects like convulsions, or coma, tinnitus and hearing loss (Waitner, 1955).

Most of anti-inflammatory agents possess anti-pyretic activities. Tolmetin showed antipyretic action in Brewer's yeast-induced pyrexia (Sangal, 1982), tolmetin produced lesions in gastric mucosa in fasted rats in single (Shriver, et al., 1975) or multiple doses (Mann, 1977; Wong et al, 1973).

In clinical trials, superiority of tolmetin in comparison to aspirin, has been found to be controversial because multicentre studies (April et al, 1975; Bain et al, 1975) observed no significant difference between two drugs.

Indomethacin was found to be more potent in inhibiting platelets as it decreased the release of platelet bound C-serotin (Zucker and Peterson, 1970). Tolmetin stored only little effect on platelet adhesiveness unlike aspirin (Sangal, 1982). However, aspirin was found to possess fibrinolytic activity and slightly prolonged coagulation and prothrombin times (Rishi et al, 1976). The haemopoietic effects of indomethacin included neutropenia, thrombocytopenia and rarely aplastic anemia, while ibuprofen also failed to modify platelet function and to

prolong bleeding time (Goodman and Gilman, 1980)

Aspirin induced hyperglycemia and hypoglycemia in certain diabetic patients (Reid et al., 1957; Whitehouse, 1965). Indomethacin instead of any direct action, just inhibited the glucagon-induced hepatic glucose production (Ganguli et al., 1979) and angiotensin-induced hyperglycemia (Singh et al., 1978). However, tolmetin showed a significant hypoglycemia (Sangal, 1982). The effect of aspirin and phenylbutazone on uric acid excretion was found to be dose dependent (Yu and Gutman, 1959; Goodman and Gilman, 1975 and Wallace et al., 1967) while tolmetin was found to decrease the serum uric acid level (Sangal, 1982).

Aspirin was shown to enhance fibrinolytic activity (Rishi et al., 1976). However, Done (1960) reported inhibition of fibrinolysis with anti-inflammatory agents in vitro.

MATERIAL & METHODS

EXPERIMENTAL STUDY

Material :

A. Animals :

i) Rabbits : Healthy adult albino rabbits of either sex, weighing between 1 to 2 kg of body weight, were used for biochemical and haematological studies.

ii) Rats : Anti-inflammatory and ulcerogenic activity of drugs was studied in adult albino rats of either sex, weighing between 100 to 200 gms of body weight.

iii) Mice : Analgesic study on acetic acid-induced writhing and toxicological study was undertaken in albino mice of either sex, weighing between 20-30 gms of body weight.

B. Drugs and chemicals :

(a) Drugs :

i) Tramaril (Unichem, Bombay) A suspension of Tramaril was freshtly prepared in 2% gum acacia just before use.

ii) Indomethacin (I.D.P.L., Hyderabad) A suspension of indomethacin was prepared in 2% gum acacia just before use.

iii) Aspirin (Jaychem, Bombay) It was also freshly prepared as a suspension in 2% gum acacia for each experiment.

iv) Tolmetin Sodium (McNeil Laboratories, Washington) An aqueous solution was prepared freshly in distilled water just before use.

v) Brufen (Boots) It was used as a suspension in 2% gum acacia freshly prepared just before experiment.

(b) Chemicals :

For pharmacological studies :

i) Acetic acid - 1% solution of acetic acid was prepared in normal saline.

ii) Carrageenin - A 1% suspension of carrageenin in 2% gum acacia in normal saline was prepared just before the experiment.

Biochemical studies :

All chemicals of Analar (BDH) grade were used.

(1) For Blood Sugar :

a) Standard Glucose Solution :

A stock solution of 15 mg% of glucose was prepared in 0.2% Benzoic acid. It was stored in the fridge at the temperature of 4°C. The solution was diluted 20 minutes at the time of estimation.

b) Alkaline Copper Reagent :

40 gms of anhydrous sodium carbonate was dissolved in 400 ml of distilled water. 7.5 gms of tartaric acid was added to it and when the tartaric acid dissolved, then 4.5 gm of crystalline copper sulphate was added and mixed thoroughly. Finally the volume was made upto 1000 ml.

c) Phosphomolybdic Acid Solution :

35 gms of molybdic acid and 5 gms of sodium tungstate were dissolved in 200 ml of 10% sodium hydroxide. It was boiled vigorously till ammonia

smell disappeared and cooled at room temperature. The volume was then made upto 350 ml by adding concentrated (85%) phosphoric acid. The final volume was made upto 500 ml with distilled water.

d) Sodium Tungstate Solution :

10 gms of sodium tungstate was dissolved in 100 ml of distilled water and was stored in glass stoppered bottle.

e) Sulphuric Acid :

0.67 N solution was prepared in distilled water and was kept in glass stoppered bottle.

(2) For Serum Uric Acid :

a) Sodium Tungstate -

10% solution.

b) Sulphuric Acid -

0.67 N solution.

c) Uric Acid Reagent -

100 gms of sodium tungstate and 20 gms of anhydrous disodium hydrogen phosphate were dissolved by heating in 150 ml of distilled water in a beaker. In another beaker 24 ml of conc. sulphuric acid was diluted with 75 ml of distilled water. Both the solutions were mixed together and poured into a flask while shaking vigorously. This resulting solution was boiled gently for 1 hour under a reflux condenser and the volume was made upto 1000 ml.

d) Sodium Cyanide (L.R. Lab. Chem.) -

12% of solution was prepared in distilled water.

e) Urea -

50% aqueous solution of urea was used.

f) Stock Standard Uric Acid Solution -

60 mg of lithium carbonate (Analar BDH) was dissolved in 15 to 20 ml of distilled water. The solution was heated to 60°C and 100 mg of uric acid (Reidel, Hungary) was added to it and stirred vigorously till it dissolved. Subsequently it was transferred to a 100 ml flask. 20 ml of 40% formalin was added to the solution and then 1 ml of 50% V/V acetic acid was added slowly with continuous stirring. The final volume was then made up to 100 ml with distilled water.

g) Standard for use -

1 ml of stock standard uric acid solution was diluted to 500 ml with distilled water just before use.

For Haematological Studies :

(1) Platelet Count -

a) Diluting fluid (Formal citrate solution) -

A solution of 1% formaline was prepared in 34.3 gm/l of trisodium citrate.

b) Ethylene diamine tetramide -

A solution of 10 mg/ml was prepared in distilled water.

c) Plasma fibrinogen -

i) Normal saline -

0.9% solution of sodium chloride.

ii) Sodium citrate -

3.8% solution in distilled water.

d) Calcium chloride -

2.5% solution of anhydrous calcium chloride in distilled water was used.

e) Acetone -

Euglobulin Clot Lysis Time :

a) Sodium citrate -

3.8% solution was prepared in distilled water.

b) Calcium Chloride -

0.276% solution in distilled water was used.

c) Acetic acid -

1% solution of acetic acid was prepared.

d) Borate solution -

9 gms of sodium chloride and 1 gm of sodium borate were dissolved in 1 litre of distilled water in order to give a pH of 9.0

Methods :

Analgesic activity :

Analgesic effect of anti-inflammatory drugs like aspirin, indomethacin, brufen, tolmetin and tromexil, were

evaluated in albino mice by the method of Acetic acid-induced writhing (Collier et al., 1968). In this study 10 ml/kg of 1% acetic acid solution in normal saline was injected intraperitoneally in six groups consisting of 10 mice each. The animals were observed for writhingwhich was characterised by a wave of constriction and elongation traversing caudally along the abdominal wall often accompanied by extension of hind limbs. Mice were pretreated with drug 30 minutes before. One group of mice was treated orally with 2 ml/kg of distilled water which serve as control while the other five groups received aquous solution of tolmetin (20 mg/kg, 50 mg/kg, 100 mg/kg) and suspension of tramaril (100 mg/kg, 150 mg/kg, 200 mg/kg), indomethacin (2 mg/kg, 5 mg/kg, 10 mg/kg), aspirin (20 mg/kg, 40 mg/kg, 80 mg/kg), brufen (10 mg/kg, 20 mg/kg, 30 mg/kg), respectively, in 2% gum acacia. Absence of writhing indicated the analgesic activity of drug.

Anti-pyretic Action :

The antipyretic activity was assessed by the method of T.A.B. vaccine - induced pyrexia (Saxena, 1979). In this method 6 groups consisting of 6 albino rabbit in each were used. One group serving as control was treated with 2 ml/kg of distilled water ad libitum and other five groups received aspirin, tramaril, indomethacin, brufen orally in a volume of 2 ml/kg in doses of 50 mg/kg, 100 mg/kg, 200 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg, 250 mg/kg, 5 mg/kg, 10 mg/kg and 10 mg/kg, 30 mg/kg and 40 mg/kg respec-

tively as a suspension in 2% gum acacia and tolmetin (25 mg/kg and 50 mg/kg) as a aqueous solution.

The normal rectal temperature of a group of rabbits was recorded by a clinical thermometer by introducing it 2 cm deep into the rectum at hourly intervals for a period of 4 hours. It was observed that in normal healthy rabbits the variations in the body temperature was minimal from 11 AM to 2 PM, therefore, the antipyretic activity of drugs was studied during this period. T.A.B. vaccine was administered intravenously into the marginal ear vein of rabbit in a dose of 0.5 ml/rabbit. The temperature was recorded every 30 minutes until it approached the normal. In the control group of rabbits, it was found that peak pyrexia was attained between 60 minutes and 90 minutes of administration of T.A.B. vaccine and the body temperature returned to normal after 4 hours. In view of this observation, it was decided to inject the drugs after 60 minutes of the vaccine administration and likewise the rectal temperature was recorded every 30 minutes till recovery.

Anti-inflammatory Activity :

The anti-inflammatory effect was studied by carrageenin-induced rat hind paw oedema (Winter et al., 1962). In order to evaluate the anti-inflammatory activity, three dose levels of each drug was used in each group. The study was undertaken in six groups of albino rats of either sex consisting of 10 rats in each group, weighing

between 150-200 gm. One group treated with 2 ml/kg of distilled water served as control while the remaining five groups were treated with tromarit (100 mg/kg, 150 mg/kg, 200 mg/kg), aspirin (50 mg/kg, 100 mg/kg, 150 mg/kg), indomethacin (1 mg/kg, 2 mg/kg, 5 mg/kg), brufen (10 mg/kg, 150 mg/kg, 20 mg/kg) and aqueous solution of tolmetin (30 mg/kg, 50 mg/kg, 100 mg/kg).

The inflammation was induced by injecting 0.1 ml of 1% carrageenin suspension subcutaneously into the planter aponeurosis of the right hind paw. Marked and measurable oedema developed after three hours of injection. The paw volume was measured by a plethysmometer (Buttle et al., 1957). The difference between the volume before and 3 hours after carrageenin injection was taken as the volume of paw oedema. Drug was administered orally, 1 hours prior to carrageenin injection. The % inhibition of oedema volume (% anti-inflammatory effect) was calculated by the following formula :

$$\left(1 - \frac{Vt}{Ve}\right) \times 100$$

where

Vt = Volume of paw swelling in treated rats.

Ve = Volume of paw swelling in control rats.

Ulcerogenic Effect :

Ulcerogenic activity of the anti-inflammatory drugs were evaluated by three methods. Each method comprised of 6 groups of six rats each. One group served as control and the other five groups were treated with anti-inflammatory

agents. The control group received 2 ml/kg of distilled water ad libitum while the other groups received 100 mg/kg 200 mg/kg, 400 mg/kg suspension in 2% gum acacia of aspirin tromaril in 100 mg/kg, 200 mg/kg, 400 mg/kg, brufen 50 mg/kg, 100 mg/kg, 200 mg/kg and indomethacin 2 mg/kg, 4 mg/kg, 6 mg/kg and aqueous solution of tolmetin (100, 200, 300 mg/kg).

(a) Ulcerogenic activity of drugs on chronic administration :

Five groups consisting of 6 rats each, were treated with drugs orally once daily for four consecutive days andone group was treated likewise with distilled water on the fifth day the rats were sacrificed under ether anaesthesia. The stomach was removed and opened along the greater curvature. It was examined with the help of a magnifying glass for the presence of ulcers. The ulcer index was calculated according to the method of Dhawan and Srimal (1973) by the following formula :

$$U\ I = \left(\frac{ADU \times Percent\ RU}{100} \right)$$

where

U I = Ulcer index

Percent RU = percentage of rats with ulceration.

ADU = Average degree of single ulceration for each group, which was determined by adding together the degree of signal ulceration (DSU) for the group divided by number of animals.

(b) Effect of drugs on experimental ulceration produced by Shay's method (Shay et al. 1945) :

Rats were anaesthetised with ether and laparotomy was done. Gastroduodenal junction was identified and ligated. The abdomen was closed with stitches. The animals were treated with distilled water or drug just after ligation. Four hours after ligation, the rats were sacrified. Stomach was removed and examined for the presence of ulcers to calculate ulcer index.

(c) Effect of drugs on stress-induced ulceration (Rossi et al., 1956) :

After 8 hours of fasting the rats were wrapped in the wire gauze to expose them to immobilization stress for a period of 4 hours. The rats were allowed distilled water or drug just before exposing them to stress. After 4 hours of stress, the rats were sacrificed and stomach was examined and ulcer index calculated.

Biochemical studies :

Blood sugar and serum uric acid levels were estimated in rabbits to assess the effect of anti-inflammatory drugs on these parameters. Each parameter was studied in 6 groups consisting of 6 rabbits each. One group received distilled water (2 ml/kg) serving as control whereas remaining five groups were treated with drugs.

(a) Collection of blood samples :

Blood samples were collected from marginal ear vein of rabbits in fluoride vials for estimation of blood sugar and in plain vials for serum uric acid estimation. After one hour, the blood samples were centrifuged (at 1000

r.p.m.) and decanted to get clear serum. Blood samples were collected just before and at hourly intervals for 4 hours and 7 days after drug treatment.

(b) Estimation of blood sugar :

The rabbits were treated with tromaxil (200 mg/kg, 250 mg/kg), indomethacin (2 mg/kg), aspirin (100 mg/kg) and brufen (10 mg/kg) as a suspension in 2% gum acacia while tolmetin (10 mg/kg) was given as an aqueous solution orally.

Blood sugar level was estimated by the method of Folin and Wu (1920). 3.5 ml of distilled water was taken in a centrifuge tube and to it was added 0.1 ml of blood in order to haemolyse thered blood cells. 0.2 ml of 10% sodium tungstate and 0.2 ml of 0.67 N sulphuric acid were subsequently added to precipitate the proteins. After mixing vigorously, it was allowed to settle down for some time and then centrifuged for 10 minutes at 1000 r.p.m. 2 ml of the supernatant was pipetted in a Folin sugar tube. A blank was prepared by taking 2.0 ml of distilled water while standard was prepared by taking 2.0 ml from the solution obtained by diluting the stock solution of standard glucose 20 times in separate test tube.

To all these test tubes, 2.0 ml of alkaline copper solution was added and kept in boiling water bath for 6 minutes and allowed to cool for 2 minutes. 2.0 ml of phosphomolybdic acid was then added to each test tube and again boiled for 5 minutes and cooled for 2 minutes. The final volume was then made upto 12.5 ml with distilled

water and mixed properly. The readings were taken immediately at 650 mu filter of the photocolorimeter.

The blood sugar level was calculated by the following formula :

$$\text{Blood sugar mg\%} = \frac{\text{Optical density of unknown}}{\text{Optical density of standard}} \times 100$$

(c) Estimation of serum uric acid :

The rabbits were treated with drugs in five groups with indomethacin (2 mg/kg), tromaril (200 mg/kg), aspirin (100 mg/kg), brufen (10 mg/kg) as a suspension in 2% gum acacia and tolmetin (10 mg/kg) as aqueous solution orally respectively.

Serum uric acid level was estimated by the method of Brown (1945). 0.1 ml of serum was added to 3.5 ml of water in a centrifuge tube. 0.2 ml of 10% sodium tungstate and 0.2 ml of 0.67 sulphuric acid were added to precipitate the proteins. After mixing vigorously it was allowed to stand for sometime and then centrifuged. 2.0 ml of supernatant was taken in a test tube. The standard and the blank were prepared by taking 2.0 ml of diluted uric acid standard and 2.0 ml of distilled water respectively in the other two tubes.

To each test tube, 2.0 ml of 12% sodium cyanide, 2.0 ml of 50% urea and 1.0 ml of phosphotungstic acid reagents were added, one by one, mixing well after each addition. It was left for 1 hour for the development of colour and finally the volume was made upto 10 ml by

adding 3.0 ml of distilled water. The readings were taken at 520 mu filter of the photocolorimeter. Serum uric acid level was estimated by using the following formula :

$$\text{Serum uric acid (mg\%)} = \frac{\text{O.D. of unknown}}{\text{O.D. of standard}} \times 8$$

Haematological studies :

Haematological studies like platelet count, clotting time, estimation of plasma fibrinogen and euglobulin clot lysis time were undertaken in rabbits.

(a) Collection of blood and drug administration for platelet count and clotting time :

Six groups of albino rabbits of 6 rabbits each of either sex were treated orally with 2 ml/kg of distilled water which served as control and remaining groups were administered indomethacin (5 mg/kg), aspirin (100 mg/kg), brufen (10 mg/kg), tromasil (200 mg/kg) as a suspension in 2% gum acacia and an aqueous solution of tolmetin (50 mg/kg).

Blood was collected from the marginal ear vein in capillary tube and pasteur pipette for clotting time and platelet count respectively.

(b) For plasma fibrinogen and euglobulin clot lysis time (E.L.T.) :

plasma fibrinogen and euglobulin clot lysis time (E.L.T.) were studied in six groups of albino rabbits of 6 rabbits each of either sex weighing between 1 to 2 kg. Animals were treated with 2.0 ml/kg of distilled water orally for 7 days in one group serving as control and the

other five groups received orally for 7 days tromaril (200 mg/kg), aspirin (100 mg/kg), brufen (10 mg/kg), indomethacin (200 mg/kg) as a suspension in 2% gum acacia and aqueous solution of tolmetin (50 mg/kg) orally respectively.

Ear of the rabbit was shaved, a sharp cut was made on marginal ear vein and blood sample was collected in a centrifuge tube containing 3.8% solution of sodium citrate 1/6 V/V of total blood collected. The blood was centrifuged and the supernatant plasma was used for both estimations. A low temperature was maintained for collection of the samples.

Platelet count :

The platelet count was done by the method of Dacie and Lewis (1975). R.B.C. pipette was rinsed with E.D.T.A. solution and the blood was taken from the marginal ear vein directly in R.B.C. pipette upto mark 1. It was diluted to 100 times by taking formal citrate diluting solution upto mark 101 of the pipette and mixed thoroughly. After discarding first few drops, the nebauer chamber was filled with the solution and was kept in a moist chamber for 20 minutes to settle the platelets. The platelets in central small square and 4 corner small squares (as in case of R.B.C. count) were counted by using 4 mm objective and X10 eye piece.

Calculation :

Platelet count/cu mm of blood = N x 5000

where N = No. of platelets in 5 squares.

Clotting time :

The clotting time was studied by the method of Best and Taylor (1966). The blood from marginal ear vein was allowed to run in capillary tube. The end of the tube were sealed by plasticin and the tubes were immersed in a water bath maintained at 37°C. At every 15 sec. interval, small fragments of tube were broken off and the end point was recorded when a string of clot was observed.

Plasma Fibrinogen :

Plasma fibrinogen was estimated by the method of Sasana et al; (1979). 1.0 ml of citrate plasma was added to 10 ml of physiological saline (0.9%) solution in a small beaker and the mixture was allowed to clot by addition of 1.0 ml of 2.5% calcium chloride and incubated at 37°C in a water bath. When clotting was complete, the clot was tipped into the palm of hand and the fluid was extruded out by gentle pressure until the clot was small enough to be squeezed between the fingers and resulted into a compact ball of fibrin. This fibrin ball was kept for 30 minutes in distilled water and then for 30 minutes in acetone and allowed to dry in a hot air oven. Then after cooling at room temperature it was weighed on a sensitive analytical balance.

Calculation :

$$\text{Plasma fibrinogen in mg\%} = \text{Dry weight of fibrin ball} \times 100$$

Euglobulin clot lysis time (E.L.T.) :

Plasma E.L.T. was determined by the method of Ducktail

(1958). 0.5 ml of plasma was added in 9.0 ml of distilled water in a glass test tube. The pH was adjusted to 5.3 by adding 0.1 ml of 1% acetic acid. The tubes were kept for 30 minutes in a refrigerator at 4°C for the euglobulin fraction of the plasma to precipitate and then centrifuged for 5 minutes at 3000 r.p.m. The supernatant was decanted and the tubes were stirred gently until the euglobulin fraction was completely dissolved in borate solution. 0.5 ml of 0.276% calcium chloride was then added to the solution of euglobulin in borate and the time, at which mixture clot was observed, was recorded. When the tubes were incubated at 37°C and examined at frequent intervals to see the lysis. When the lysis was almost complete, the clot was observed every five minutes for accurate measurement. Mean time taken between the clot formation and its complete lysis was recorded as the euglobulin clot lysis time.

Acute toxicity study (Ghosh, 1971) :

Acute toxicity was studied in 5 groups of albino mice of either sex consisting of 10 mice in each group, weighing between 25 to 40 gm. All the animals were kept on standard laboratory diet. 2% suspension in gum acacia of tramaril, aspirin, indomethacin, brufen and aqueous solution of tolmetin were administered orally to mice in graded doses. After administration of the drug, clinical signs were observed daily for 24 hours. Values of LD_{50} were calculated on the basis of the mortality occuring during this period in a particular dose.

Statistical analysis :

The data obtained in the present study was analysed statistically by the student's 't' test. Indices and percentage inhibition were calculated by using standard procedures. Wilcoxon sign rank test was applied in non-parameteric datas.

Clinical study :

(a) Patients :

Twenty patients, suffering from 'definite' or classical rheumatoid arthritis, were selected for the clinical study and were kept under observation for treatment from July'82 to February'83. 12 female and 8 male patients were included in the study. These patients were divided into two groups - aspirin-treated which served as control group and tramaril-treated as study group. A detailed history regarding classical symptoms of the disease were recorded and patient reporting any symptom like nausea, vomiting, diarrhoea, drowsiness, burning sensation, epigastric pain, headache, insomnia, haematemesis, melaena or rash in either group was excluded from the study. Patient receiving either drug in prescribed doses was assessed daily for development of any side effect.

(b) Study design :

The study was carried out as a comparison between aspirin and tramaril in daily doses of 2400 mg and 1600 mg respectively. All the anti-inflammatory drug being used previously was withdrawn and patients were allocated to two

treatment groups. The response of the therapy was noted after two weeks and four weeks of treatment. Evaluation of digital joint (P.I.P.) circumference duration of morning stiffness, grip strength, degree of pain, walking time, fever and E.S.R. was made before the start of study, after 2 weeks and four weeks treatment period. A record of side-effects reported by the patient during treatment was also maintained. Complete haematological investigations, urine examination and stool examination were also performed initially and after 4 weeks of treatment.

METHODS :

Digital Joint (P.I.P.) Circumference :

P.I.P. circumference was studied with the help of a measuring tape by the method of Mathur et al., (1980). Measurement was recorded initially and after two weeks and four weeks of drug treatment.

Grip strength :

Assessment of grip strength was done by the ability of the patient to raise the mercury level in the sphygmomanometer by squeezing therubber ball as described by Mathur et al. (1980). The reading was noted before starting the treatment and at the end of two weeks and four weeks of therapy.

Walking time :

It was evaluated by recording the time taken by the patient to walk a distance of 30 feet (Sattur et al., 1980).

Assessment of pain :

Subjective assessment of pain was done by the method of Punjabi et al, (1980). Different scores were given depending upon the degree of severity of pain.

Fever :

The effect of aspirin and tromaril on pyrexia was evaluated by clinical thermometer. Changes in body temperature (oral) were recorded before treatment and at an interval of ½ hour, 3 hours, 6 hours and 8 hours after administration of drug. The ggrameter used for comparison willlbe :

1- Rate of variation in temperature.

2- Degree in fall of temperature.

3- Duration of fall of temperature.

E.S.R. :

It was evaluated by the method of Wintrobe (1975). Changes in E.S.R. were recorded before initiating the therapy and after 4 weeks of treatment in both aspirin and tromaril-treated groups.

Side effects :

Drug induced side effects were recorded regularly as reported by patients during the period of active drug treatment.

OBSERVATIONS & RESULTS

OBSERVATIONS AND RESULTS

In the present study experimental and clinical effects of tromaril and some commonly used and newly introduced drugs have been studied. In this study the pharmacological, haematological, biochemical and toxicological effects of tromaril were compared with other anti-inflammatory agents like - aspirin, indomethacin, tolmetin and brufen. The experimental study was conducted in mice, rats and rabbits and patients suffering from different types of arthritis were selected for clinical studies. In the clinical study the beneficial and toxic effects of tromaril were compared only with aspirin.

EXPERIMENTAL :

A. ANTI-INFLAMMATORY EFFECT :

1. Carrageenin-induced hind paw oedema : Tromaril, aspirin, indomethacin, brufen and tolmetin given in graded doses, produced a dose-dependent decrease in carrageenin-induced hind paw oedema which was statistically highly significant ($P < .001$) (Table-1, Figure 2,3), except tromaril (100 mg/kg) which did not produce any significant response. The ED_{50} and LD_{50} and safety index of the anti-inflammatory drugs are compared in Table- 9(b). Tromaril appear to be nearly equal to aspirin in potency and safety in animal studies.

2. Ulcerogenic activity :
Aspirin, brufen, indomethacin, tolmetin and tromaril, when administered orally once daily for four consecutive days in graded doses, showed significant

TABLE - 1

Comparative effect of few anti-inflammatory drugs on carrageenin-induced oedema

Sl. No.	Group	No. of animals	Dose (mg/kg)	Carrageenin-induced oedema volume (ml) (Mean ± S.E.)	% Inhibition
1.	Control (Distilled water)	10	2 ml	0.22 ± 0.98	
2.	Tromaril	10	100	0.14 ± 0.041	26.36
3.			150	0.12 ± 0.031**	45.45
			200	0.082 ± 0.008***	60.90
3.	Aspirin	10	50	0.13 ± 0.004***	40.90
			100	0.097 ± 0.005***	55.91
			150	0.07 ± 0.002***	68.18
4.	Indomethacin	10	1	0.10 ± 0.021	18.80
			2	0.15 ± 0.01***	31.81
			5	0.10 ± 0.004***	54.54
5.	Brufen	10	10	0.13 ± 0.003***	40.90
			15	0.11 ± 0.004***	50.00
			20	0.09 ± 0.002***	59.09
6.	Tolmetin	10	30	0.12 ± 0.004***	36.36
			50	0.11 ± 0.003***	50.00
			100	0.094 + 0.006***	57.27

*p ∠ .05. **p ∠ .01. ***p ∠ .001

ANTI-INFLAMMATORY EFFECT

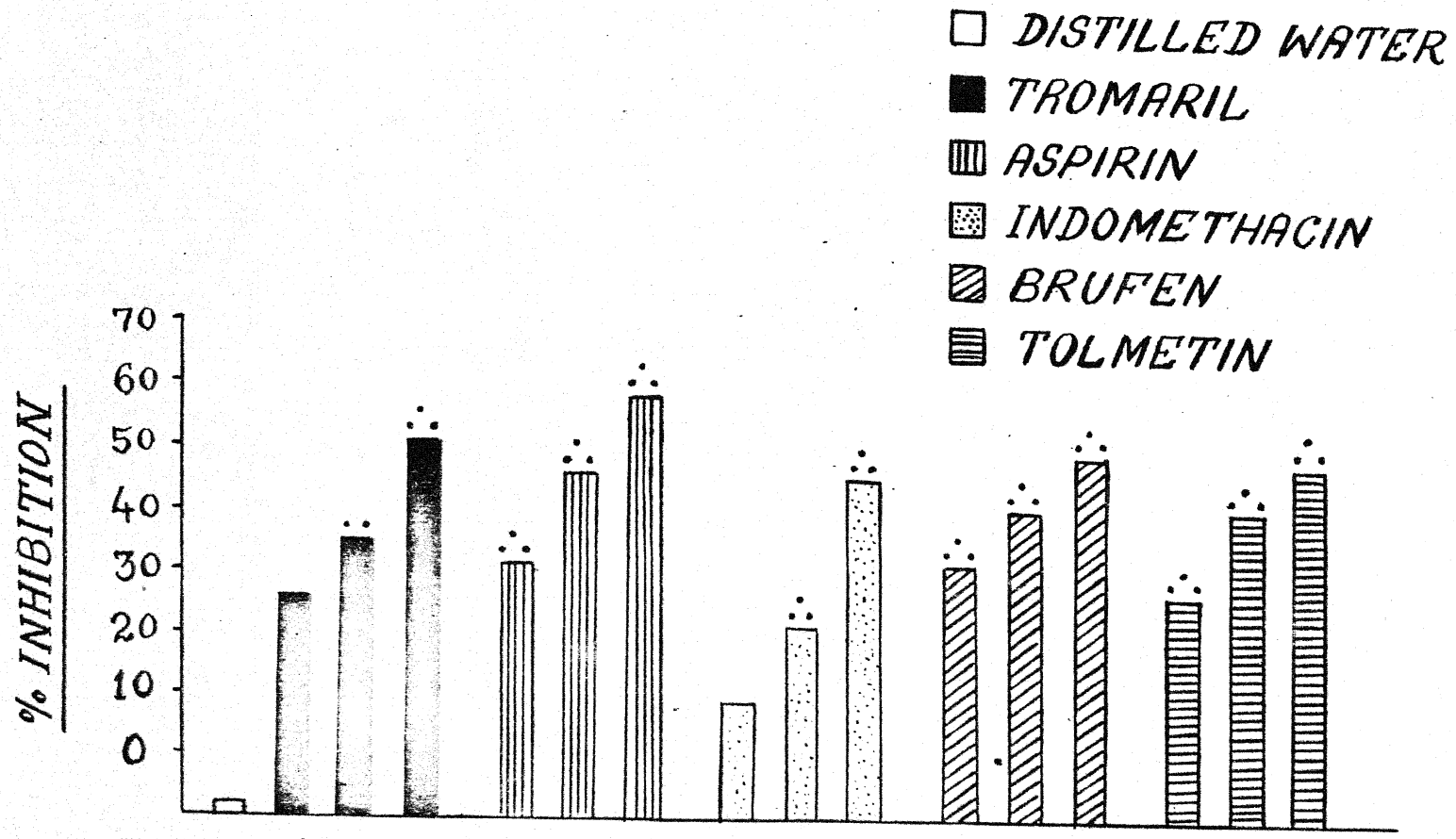


FIG. NO. 2. % INHIBITION OF CARRAGEENIN-INDUCED HIND PAW OEDEMA IN RATS WITH DISTILLED WATER (2ml/kg), TROMARIL 100mg, 200mg, 250mg/kg.), ASPIRIN (50mg, 100mg, 150mg/kg), INDOMETHACIN 1mg, 2mg, 5mg/kg.), BRUFEN (10mg, 15mg, 20mg/kg.) AND TOLMETIN (30mg, 50mg, 100mg/kg.) (·, ··, ·· ·) DENOTS P VALUS <·05, <·01, <·001 RESPECTIVELY.

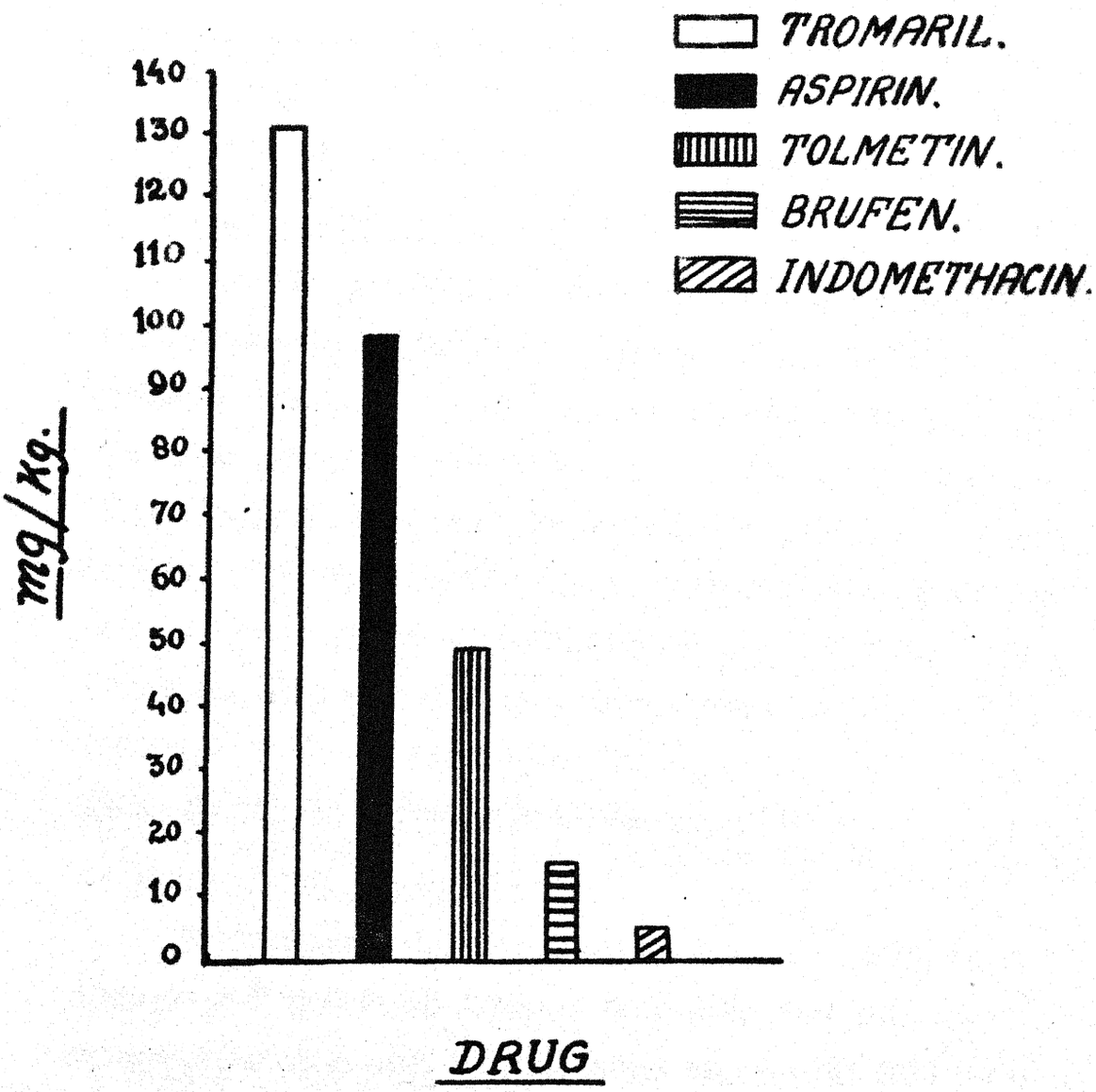


FIG. NO. 3. ANTI-INFLAMMTORY ED-50 VALUES IN RATS.

ulcerogenic effect as evidenced by high ulcer indices (Table-2, Figure 4,5,6). Aspirin, indomethacin and tolmetin produced marked increase in ulcer index in stress - induced and pyloric ligation - induced gastric ulceration. However, tromaril and brufen caused slight increase in ulcer index by Shay's technique and of stress - induced ulcer (Table-2, Figure 4,5,6).

B. ANALGESIC EFFECT :

Acetic acid (1%) in normal saline when injected intraperitoneally into mice, induced writhing in all the animals of control group. The highest dose used in this study of tromaril, aspirin, indomethacin, when administered orally in graded doses effectively protected animals whereas tolmetin and brufen prevented writhing by 80% and 70% of mice respectively. Therefore, all the drugs were effective in protecting mice from acetic acid induced writhing (Table-3, Figure 7). The ED_{50}, LD_{50} and safety index of the drug are compared in Table (9a).

C. ANTI-PYRETIC EFFECT :

Intravenous administration of T.A.B. vaccine in a dose of 0.5 ml/rabbit produced a gradual rise in body temperature with peak pyrexia after 60 minutes following vaccine administration. The body temperature returned to normal after 4 hours. Aspirin, indomethacin, tolmetin and brufen given in graded doses produced statistically significant decrease in T.A.B. vaccine - induced pyrexia. However, tromaril (100 mg/kg) showed a weak antipyretic

TABLE - 2

Comparative ulcerogenic effect of anti-inflammatory drugs in rats

Sl. No.	Group	No. of animals	Dose (mg/kg P.O.)	4 days treatment of drug			Effect on Shay's ulceration			Effect on stress ulceration		
				A.D.U.	% RU	UI	A.D.U.	% RU	UI	A.D.U.	% RU	UI
1.	Control (Distilled water)	6	2 ml	0.25	40	0.1	2.2	100	2.2	1.2	75	0.90
2.	Aspirin	6	100	1.33	66.66	0.88	2.8	87.5	2.45	0.2	83.33	1.6
			200	2.5	100	2.5	3.2	80	2.56	3.5	75	2.62
			400	3.66	100	3.66	4.0	100	4.00	5.01	75	3.75
3.	Tremaril	6	100	0.0	0.0	0.0	2.2	100	2.2	1.2	83.33	0.99
			200	0.41	66.66	0.27	2.46	100	2.4	1.5	75	1.12
			400	1.25	100	1.25	3.41	83.33	2.84	2.5	100	2.50
4.	Brufen	6	50	0.0	0.0	0.0	2.5	100	2.5	1.6	70	1.12
			100	0.41	100	0.41	2.67	100	2.6	1.8	80	1.44
			200	1.00	100	1.0	2.83	100	2.8	3.0	70	2.40
5.	Indomethacin	6	2	1.2	80	0.96	2.24	100	2.24	1.2	100	1.2
			4	2.9	100	2.9	3.60	100	3.60	2.9	1.00	2.9
			6	3.0	100	3.0	3.90	100	3.9	3.4	100	3.4
6.	Tolmetin	6	100	1.5	40	0.74	2.5	87.5	2.45	1.0	100	1.00
			200	2.8	100	2.8	2.6	87.5	3.15	3.35	87.5	2.93
			300	3.5	100	3.5	3.8	100	3.8	4.00	100	4.00

ADU = Average degree of ulcer
%RU = Percentage of rats with ulcer
UI = Ulcer index

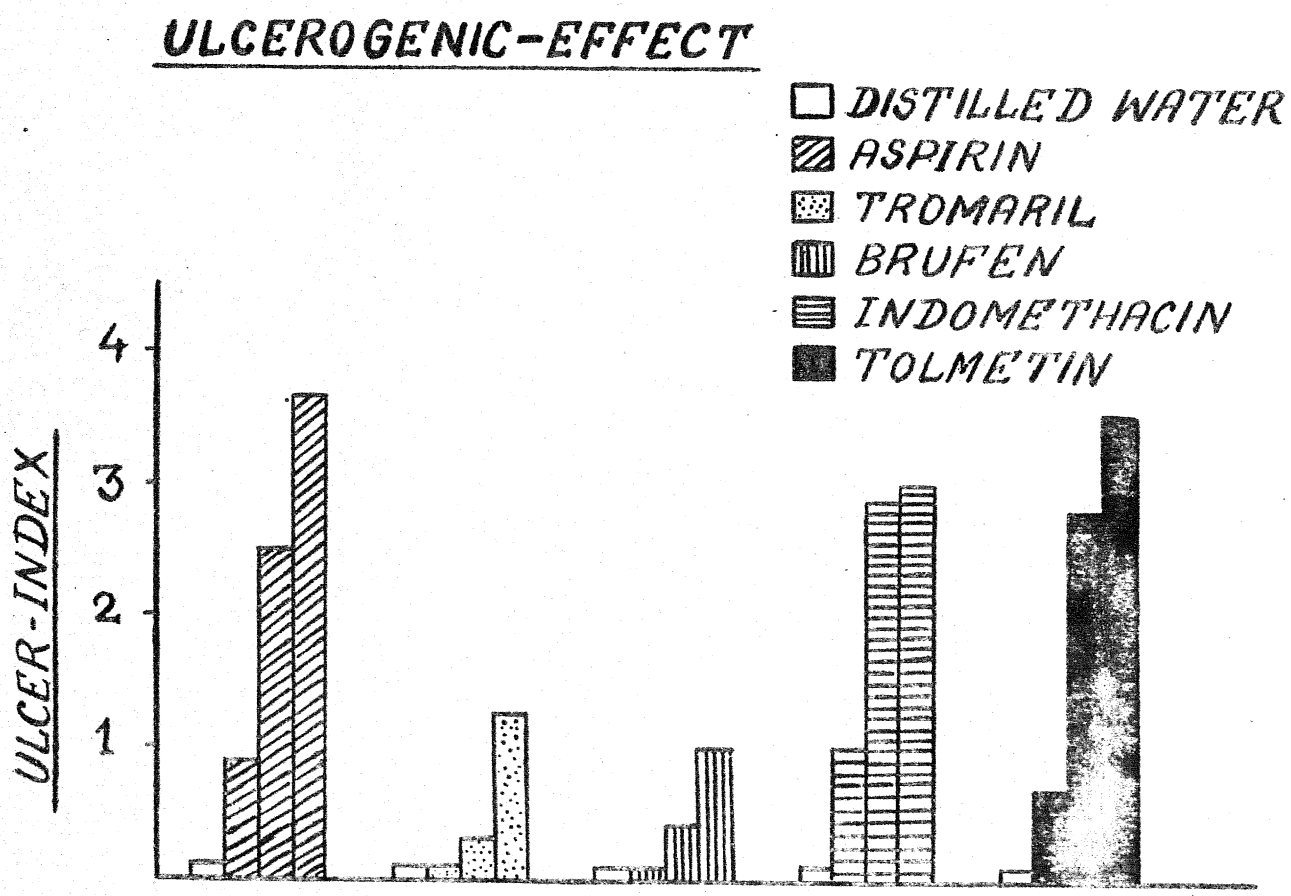


FIG. No. 4. PER SE EFFECT OF 4 DAYS TREATMENT WITH DISTILLED WATER (2 ml/kg), ASPIRIN (100 mg, 200 mg, 400 mg/kg), TROMARIL (100 mg, 200 mg, 400 mg/kg.), BRUFEN (50 mg, 100 mg, 200 mg/kg), INDOMETHACIN (2 mg, 4 mg, 6 mg.) AND TOLMETIN (100 mg, 200 mg, 300 mg/kg.) ON ULCER-INDEX.

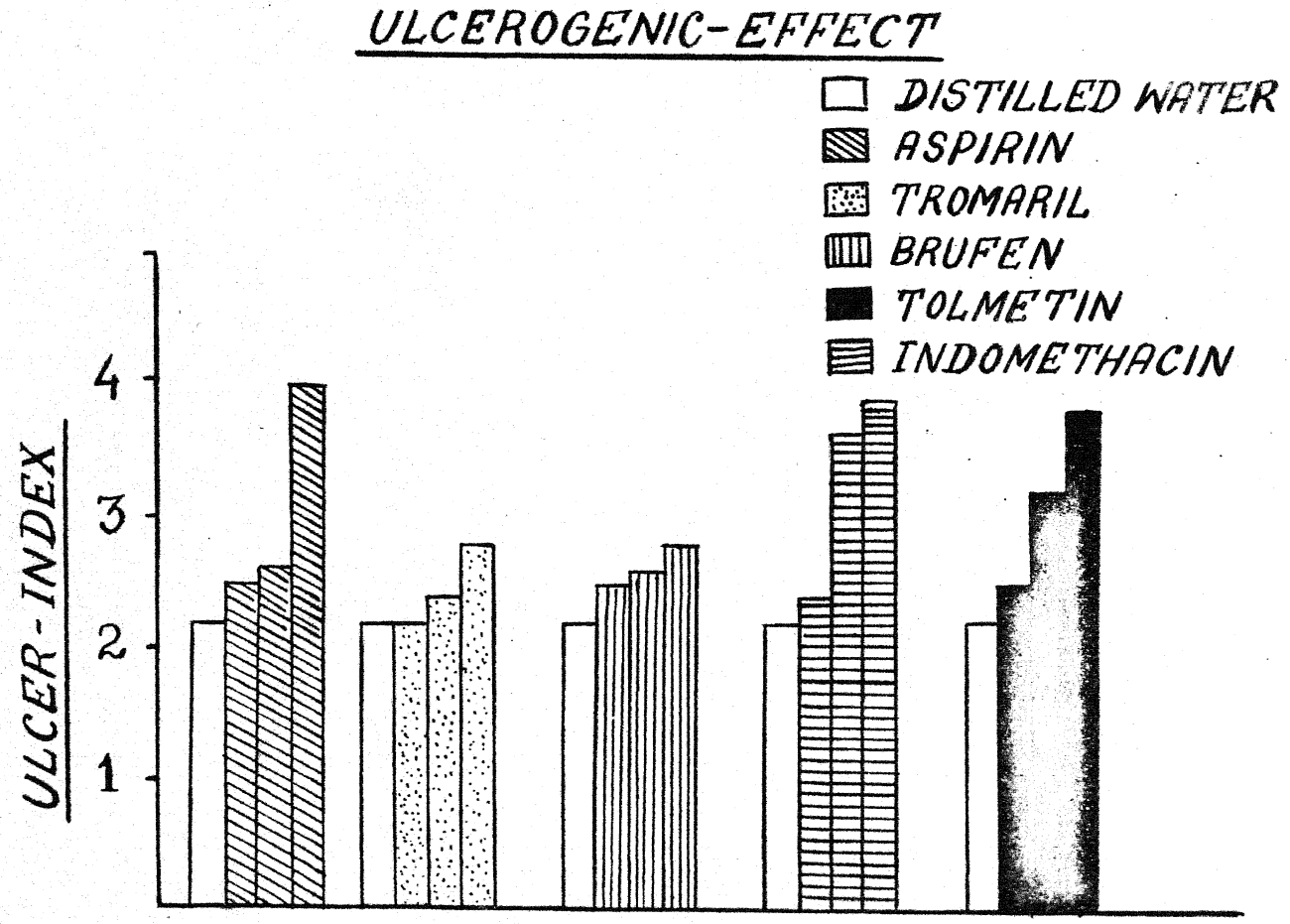


FIG.No.5. EFFECT OF DISTILLED WATER (2ml/kg), ASPIRIN (100mg, 200mg, 400mg/Kg.), TROMARIL (100mg, 200mg, 400mg/Kg.), BRUFEN (50mg, 100mg, 200mg/Kg.), INDOMETHACIN (2mg, 4mg, 6mg/Kg.) AND TOLMETIN (100mg, 200mg, 300mg/Kg) ON ULCER-INDEX IN PYLORIC LIGATION INDUCED ULCERS IN RATS.

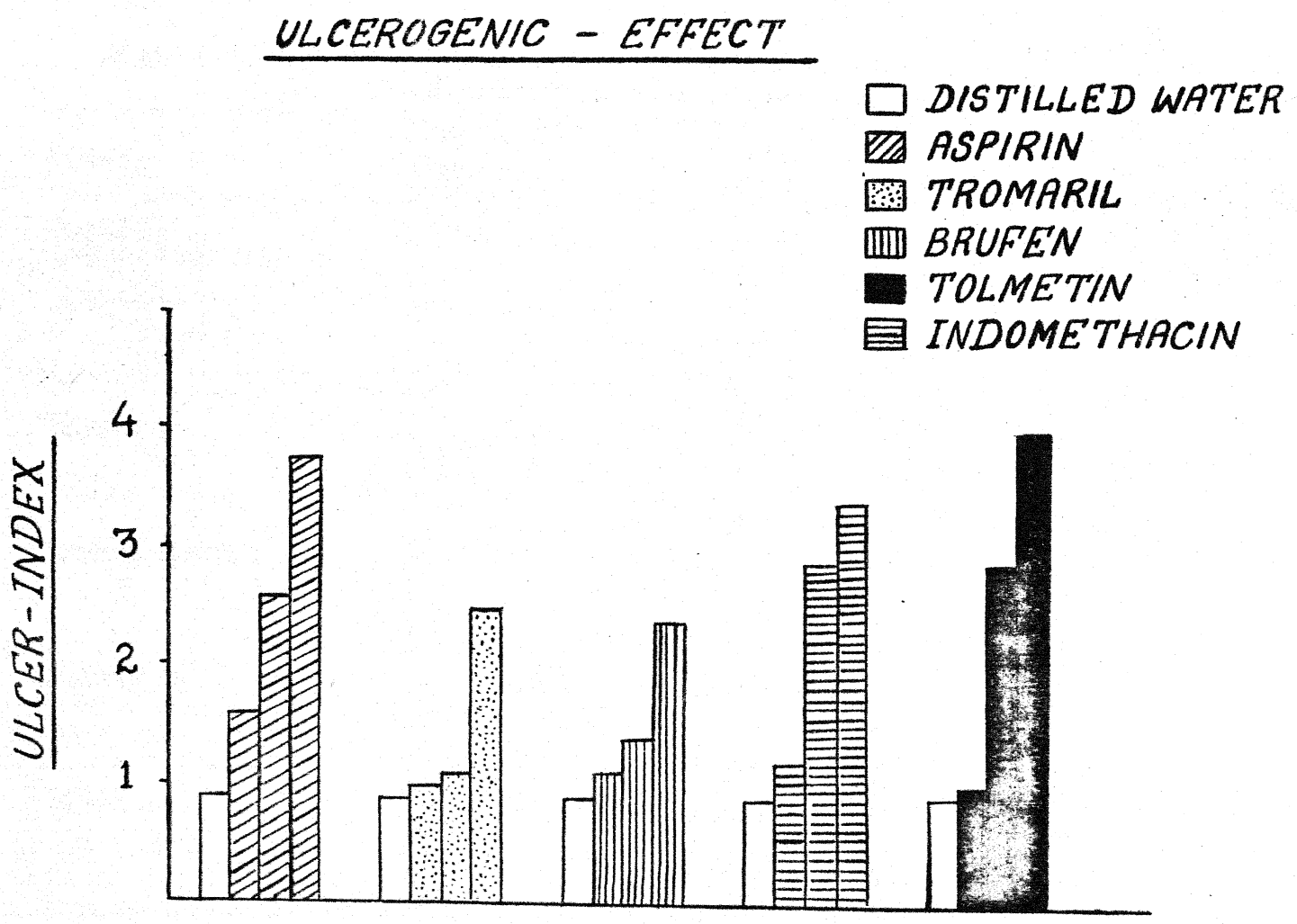


FIG. NO. 6. EFFECT OF DISTILLED WATER (2ml/kg), ASPIRIN (100 mg, 200 mg, 400 mg) TROMARIL (100 mg, 200 mg, 400 mg/kg.), BRUFEN (50 mg, 100 mg, 200 mg/kg.), INDOMETHACIN (2 mg, 4 mg, 6 mg/kg.), AND TOLMETIN (100 mg, 200 mg and 300 mg/kg.) ON ULCER-INDEX ON STRESS-INDUCED ULCERS IN RATS.

TABLE - 3

Comparative effect of Tramaril, Aspirin, Indomethacin, Tolmetin and Brufen on acetic acid-induced writhing

Group	No. of animals tested for writhing	Dose (mg/kg)	No. of animals protected	Analgesic activity (% protection)
Control (Distilled water)	10	2 ml	-	0
Tramaril	10	100	0	0
		150	6	60
		200	10	100
Aspirin	10	30	4	40
		40	8	80
		50	10	100
Indomethacin	10	2	3	30
		5	5	50
		10	10	100
Tolmetin	10	20	3	30
		50	4	40
		100	8	80
Brufen	10	10	4	40
		20	6	60
		30	7	70

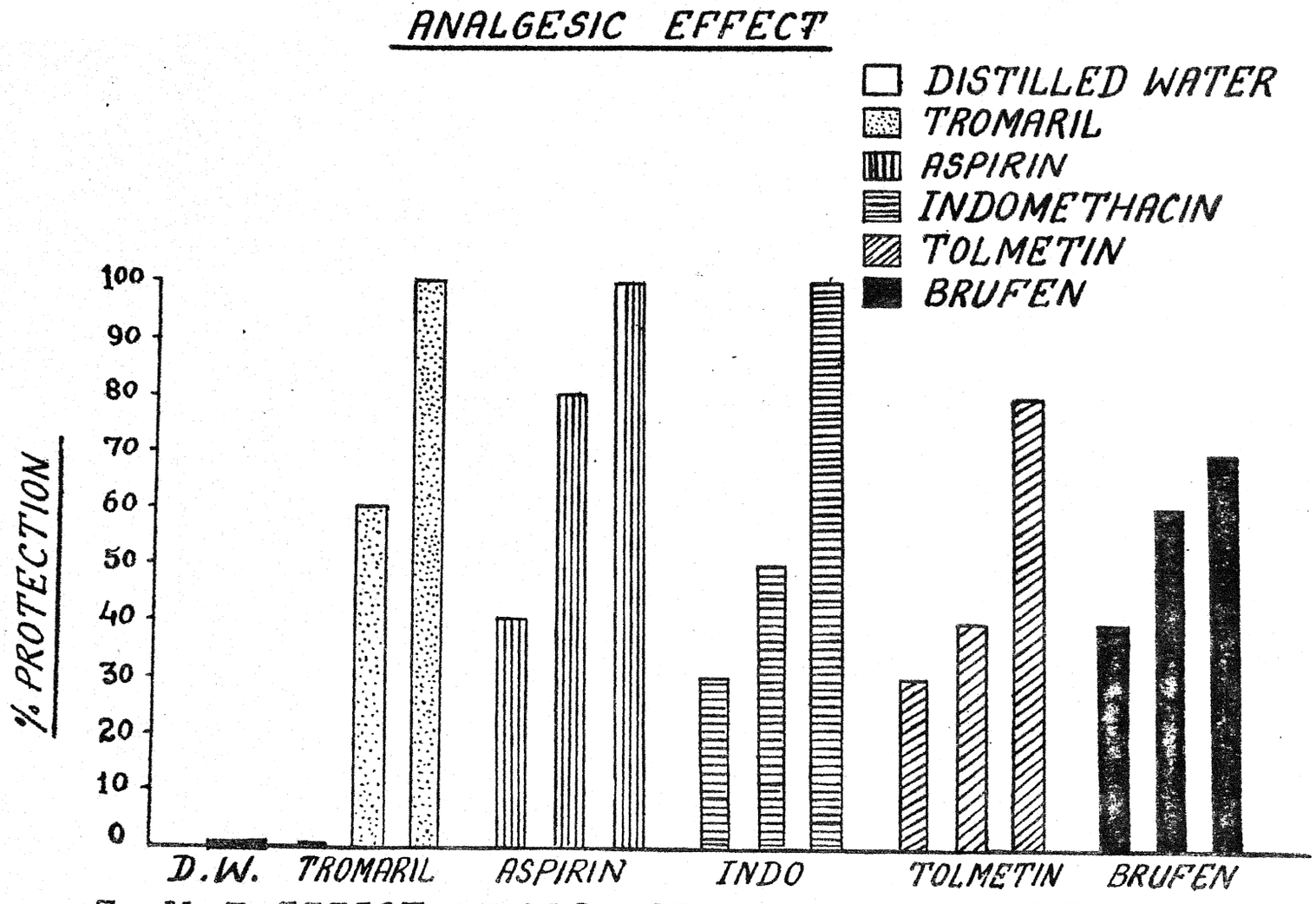


FIG. NO. 7. EFFECT OF ORAL ADMINISTRATION OF DISTILLED WATER (D.W. 2 ml/kg.), TROMARIL (100 mg, 150 mg, 250 mg/kg.), ASPIRIN (20 mg, 40 mg, 50 mg/kg.), INDOMETHACIN (INDO, 2 mg, 5 mg, 10 mg/kg.), BRUFEN (10 mg, 30 mg, 40 mg/kg.) AND TOLMETIN (20 mg, 50 mg, 100 mg/kg.) ON ACETIC ACID-INDUCED [illegible] IN MICE.

TABLE - 4

Time course effect of few anti-inflammatory drugs on TAB vaccine-induced pyrexia in rabbits

Group Dose mg/kg	Before treatment	Increase body temperature (C°) mean ± S.E. after TAB vaccine 0.5 ml/rabbit							
		30	60	90	120	150	180	210	240
Control (2ml/kg) (Distilled water)	39.54±.090	0.75±0.04	1.19±0.06	1.16±0.04	0.88±0.09	0.66±0.12	0.46±0.13	0.36±0.1	0.22±0.01
TROMARIL									
100	39.03±81	0.66±.07	1.09±.08	0.96±.09	0.77±.09	0.52±0.76	0.37±0.18	0.31±0.13	0.27±0.12
150	39.13±0.12	0.66±0.14	1.20±0.17	0.87±0.22	0.77±0.18	0.51±0.17	0.46±0.19	0.36±0.19	0.21±0.12
200	39.11±0.11	0.67±0.14	1.03±0.18	0.59±0.17*	0.33±0.11*	0.32±0.09	0.20±0.09	0.20±0.09	0.20±0.09
250	38.83±0.14	0.65±0.42	1.001±0.09	0.48±0.08*	0.36±0.10*	0.37±0.11	0.30±0.11	0.27±0.12	0.28±0.12
ASPIRIN									
50	38.79±0.29	0.70±0.05	1.00±0.07	0.85±0.15	0.70±0.12	0.56±0.08	0.35±0.07	0.30±0.07	0.28±0.16
100	38.92±0.17	0.66±0.15	1.01±0.04	0.46±0.12*	0.40±0.10*	0.39±0.10	0.31±0.09	0.29±0.07	0.27±0.13
200	38.75±0.16	0.67±0.03	0.99±0.07	0.48±0.14*	0.35±0.12*	0.37±0.07*	0.34±0.04	0.32±0.07	0.28±0.10
BRUFEN									
10	38.90±0.11	0.50±0.09	0.95±0.07	0.85±0.12	0.62±0.19	0.57±0.16	0.49±0.17	0.39±0.12	0.33±0.11
30	38.92±0.06	0.59±0.05	0.96±0.10	0.81±0.10	0.49±0.08*	0.38±0.04*	0.35±0.04	0.25±0.04	0.20±0.04
40	38.96±0.07	0.66±0.12	1.02±0.16	0.62±0.04*	0.35±0.05*	0.31±0.05*	0.27±0.09	0.28±0.05	0.23±0.03
TOLMETIN									
25	38.78±0.09	0.62±0.08	1.09±0.07	0.86±0.10	0.48±0.08*	0.37±0.08	0.37±0.05	0.24±0.12	0.20±0.04
50	38.90±0.11	0.67±0.04	0.92±0.16	0.39±0.04**	0.30±0.05**	0.21±0.03**	0.18±0.06*	0.12±0.03	0.22±0.07
INDOMETHACIN									
2	39.13±0.07	0.62±0.05	0.98±0.02	0.57±0.07*	0.42±0.17*	0.36±0.06*	0.29±0.07	0.25±0.01	0.22±0.14
5	39.11±0.08	0.68±0.12	1.002±0.06	0.31±0.06**	0.42±0.07**	0.25±0.12*	0.23±0.05	0.23±0.02	0.22±0.13

* ∠ 0.05 ** ∠ 0.01 *** ∠ 0.001

ANTIPYRETIC - EFFECT

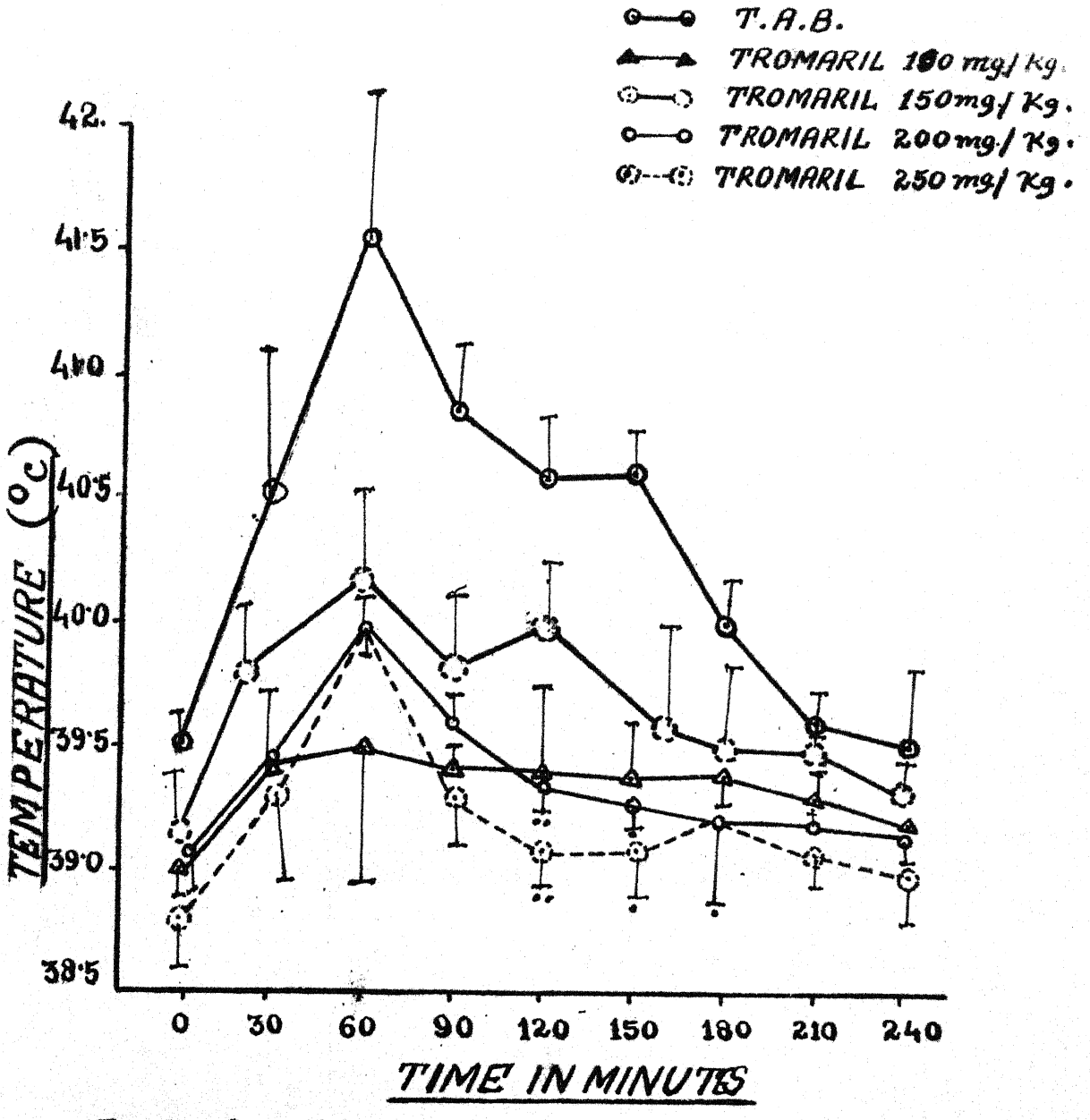


FIG. NO. 8. EFFECT OF ORAL ADMINISTRATION OF TROMARIL (100 mg/Kg.), 150 mg/Kg., 200 mg/Kg., 250 mg/Kg) ON T.A.B. VACCINE-INDUCED PYREXIA IN RABBITS.
(•, ••), DENOTES P VALUES <.05, <.01, RESPECTIVELY.

ANTIPYRETIC-EFFECT

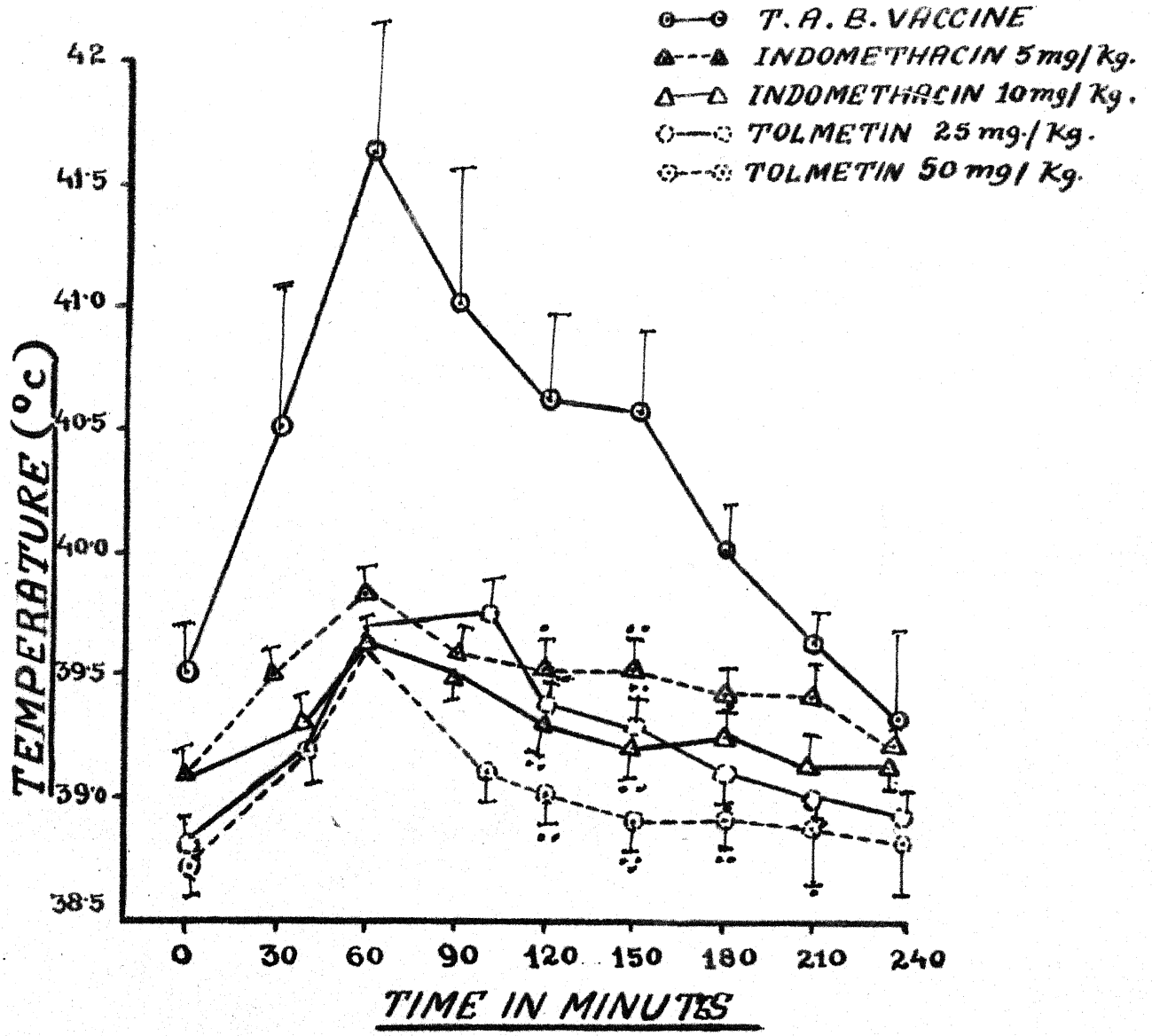


FIG. NO. 9. EFFECT OF ORAL ADMINISTRATION OF INDOMETHACIN (5mg, 10mg./Kg.) AND TOLMETIN (2mg, 50mg/Kg.) ON T.A.B. VACCINE-INDUCED PYREXIA IN RABBITS. (•, ••), DENOTES P VALUES <.05, <.01, RESPECTIVELY.

ANTI-PYRETIC-EFFECT

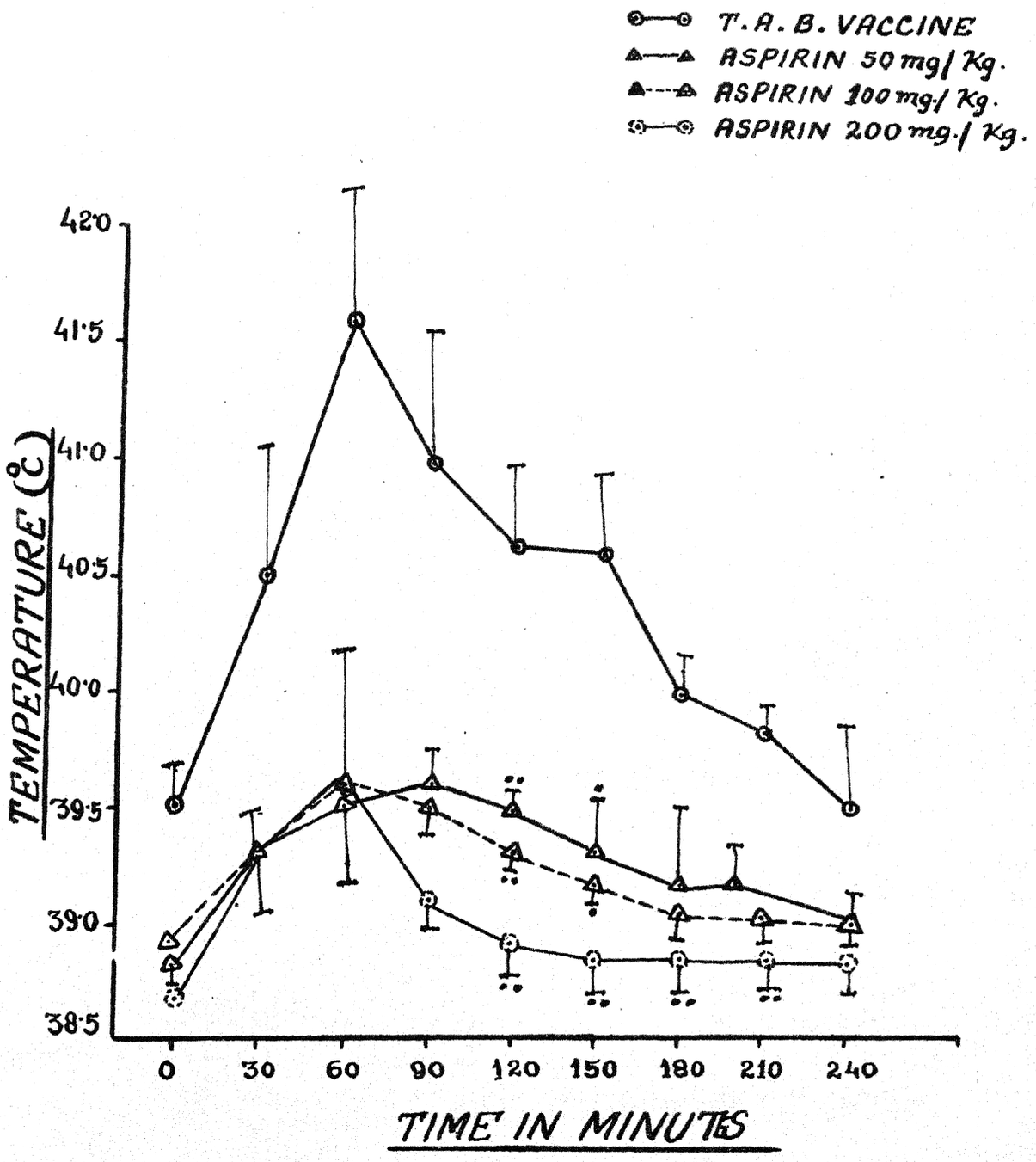


FIG. NO. 10. EFFECT OF ORAL ADMINISTRATION OF ASPIRIN (50 mg, 100 mg, 200 mg./Kg.) ON T.A.B. VACCINE-INDUCED PYREXIA IN RABBITS.
(•,••) DENOTES P VALUES <.05, <.01 RESPECTIVELY.

ANTI-PYRETIC EFFECT

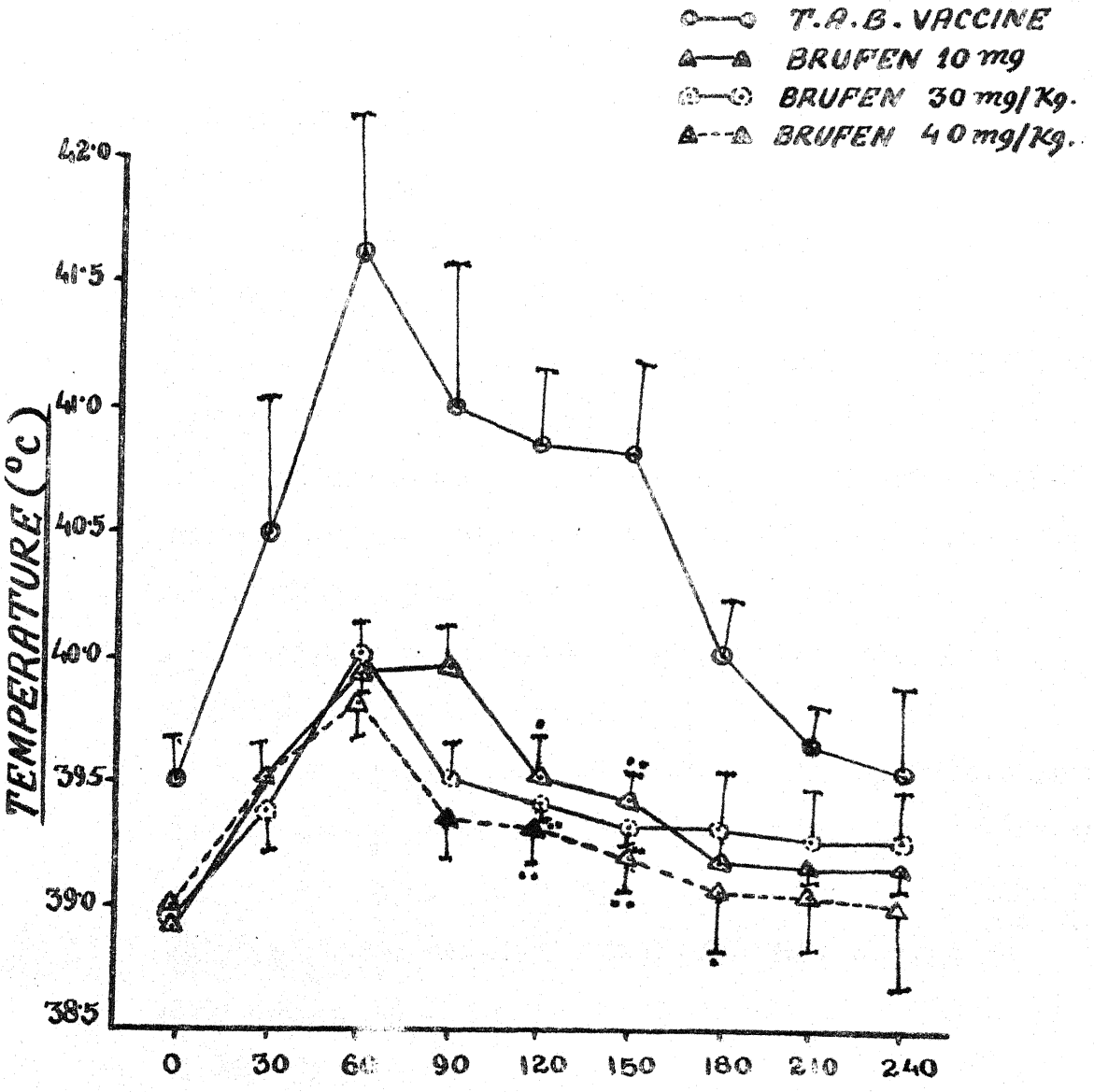


FIG. NO. 11. EFFECT OF ORAL ADMINISTRATION OF BRUFEN (10 mg, 30 mg, AND 40 mg/Kg.) ON T.A.B. VACCINE INDUCED PYREXIA IN RABBITS. (•, ••), DENOTES P VALUES <.05, <.01, RESPECTIVELY.

effect while magnitude of antipyretic activity was statistically significant in a dose of 150 mg/kg, 200 mg/kg and 250 mg/kg (Table-4, Figure 8,9,10,11).

D. HAEMATOLOGICAL STUDIES :

1. Effect on blood coagulation time :

In the control group of rabbits, the normal blood coagulation time was found to be 147.3±0.34 seconds. Distilled water per se did not produce any change in coagulation time. The clotting time observed after 24 hours and 7 days following administration of distilled water were 147.46±0.43, 147.9±0.40 seconds, respectively. Indomethacin (5 mg/kg), brufen (10 mg/kg) and tolmetin (50 mg/kg) produced highly significant decrease in coagulation time ($P < .001$). Aspirin (100 mg/kg) produced a statistically highly significant decrease ($P < .001$) in coagulation time after 24 hours and 7 days of drug administration. However, tromarli (200 mg/kg) did not show any significant change in coagulation time during the observation period (Table-5, Figure 12).

2. Effect of blood platelet count :

In control group, the platelet count was found to be 262.81±0.41 thousand/cumm of blood. On oral administration of distilled water in the albino rabbits, no change was observed in the blood platelet count. Indomethacin (5 mg/kg), tolmetin (50 mg/kg) and aspirin (100 mg/kg) was found to decrease the blood platelet

TABLE - 5

Comparative effect of four anti-inflammatory drugs on clotting time and platelet count

Drug (Dose mg/kg)	Clotting time (in seconds) (Mean & S.E.)			Platelet count (in thousand/cumm of blood) Mean ± S.E.		
	Before treatment	After treatment 24 hours	7 days	Before treatment	After treatment 24 hours	7 days
Control (2ml)	147.3±0.034	147.46±0.43	147.9±0.41	248.81±0.41	249.85±1.33	249.03±2.2
Indomethacin (5)	149.13±0.013	115.36±1.***36	99.3±4.***35	247.76±0.45	198.03±0.***41	175.53±4.***65
Brufen (10)	148.93±0.63	101.36±2.9*	83.46±4.***06	250.21±0.47	245.2±2.87	237.23±1.**1
Tolmetin (50)	146.3±1.17	141.73±0.***97	122.33±1.***55	251.53±0.93	195.39±3.***1	184.68±1.***98
Aspirin (100)	146.86±0.44	135.03±0.***76	127.01±0.***95	249.5±0.32	232.11±2.***67	235.61±1.**84
Tromaril (200)	146.76±0.29	146.41±0.68	147.4±0.39	247.63±0.29	249.38±0.29	250.25±0.57

No. of animals = 6 with all drugs

*p < 0.05

**p < 0.01

***p < 0.001

CLOTTING TIME

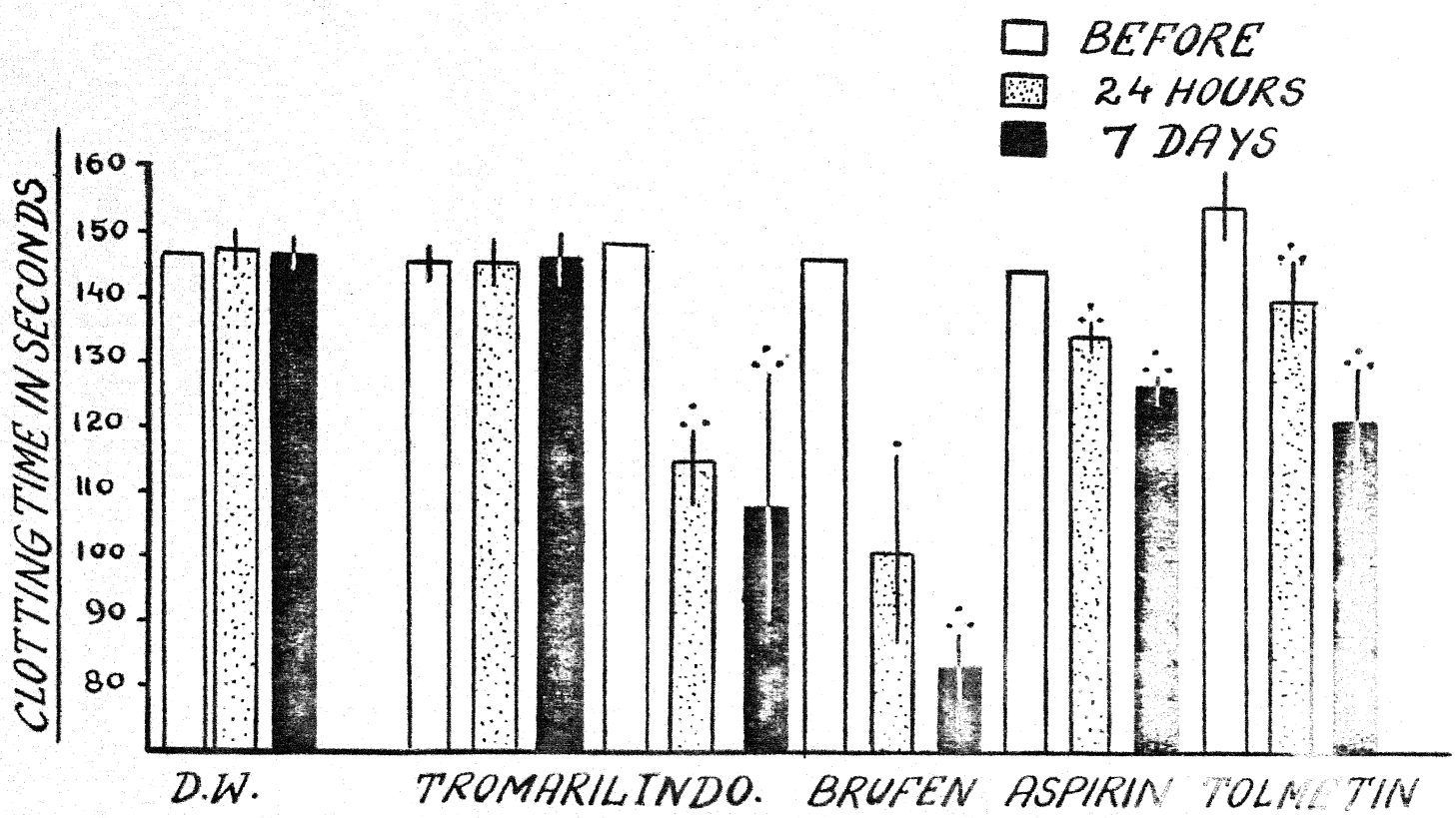


FIG. NO. 12. EFFECT OF ORAL ADMINISTRATION OF DISTILLED WATER (D.W.) 2 ml/kg, TROMARIL (200 mg/Kg.), INDOMETHACIN INDO. 5 mg/Kg.) BRUFEN (10 mg/Kg), TOLMETIN (50 mg/Kg.), AND ASPIRIN (100 mg/Kg) ON CLOTTING TIME IN RABBITS. (·, ··, ∴) DENOTES P VALUES ■, <·05, <·01, <·001 RESPECTIVELY.

PLATELET COUNT

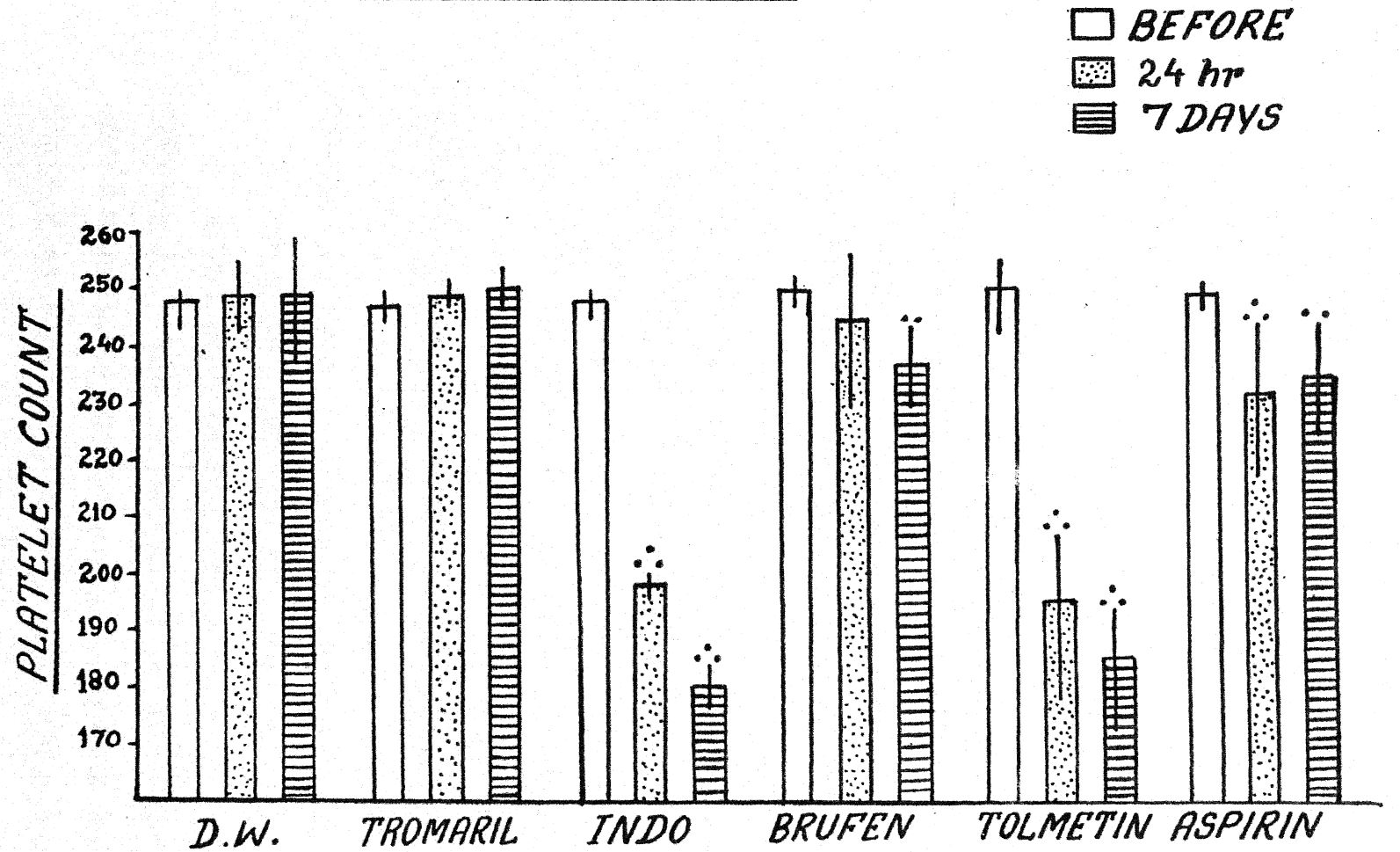


FIG. NO. 13. EFFECT OF ORAL ADMINISTRATION OF DISTILLED WATER (D.W. 2ml/kg), TROMARIL (200 mg/kg), INDOMETHACIN (IND. 5 mg/kg.), BRUFEN (10 mg/kg.), ASPIRIN (100 mg/kg.), ON PLATELET COUNT IN RABBITS. (·, ··, ∴) DENOTES P VALUES < ·05, < ·01, < ·001) RESPECTIVELY.

count significantly ($P < .001$) after 24 hours and 7 days of drug administration while brufen (10 mg/kg) did not show any significant change in platelet count but decreased it markedly after 7 days of drug treatment. However, tromarik (200 mg/kg) failed to show any change in the platelet count during this period (Table-5, Figure 13).

3. Effect on plasma fibrinogen level :

Tromaril (200 mg/kg), tolmetin (50 mg/kg), aspirin (100 mg/kg), brufen (10 mg/kg) and indomethacin (2 mg/kg) were administered orally in albino rabbits for 7 consecutive days to see their effects on plasma fibrinogen content. Plasma fibrinogen level in the control group (administered with distilled water) was found to be 204.3±1.16 mg%. Tolmetin, indomethacin and aspirin increased the plasma fibrinogen count markedly while brufen showed very slight increase in plasma fibrinogen content. These changes were statistically significant. Tromaril (200 mg/kg) failed to produce any significant change in plasma fibrinogen content (Table-6, Fiure 14).

4. Effect on euglobulin clot lysis time (E.L.T.) :

Tolmetin (50 mg/kg), indomethacin (2 mg/kg), aspirin (100 mg/kg), brufen (10 mg/kg) and tromaril (200 mg/kg) were administered orally in albino rabbits for 7 consecutive days to study their effect on plasma E.L.T. Tolmetin, indomethacin and aspirin showed significant increase in plasma E.L.T. while brufen and tromaril decreased if although this decrease in plasma

TABLE - 6

Comparative effect of few anti-inflammatory drugs on plasma fibrinogen and euglobulin clot lysis time in rabbits

Group (Dose mg/kg)	P.F. Content (mg%) (Mean ± S.E.)		Plasma E.L.T. (in minutes) (Mean ± S.E.)	
	Before treatment	After treatment	Before treatment	After treatment
Control (2ml)	204.6±1.16	206.3±1.4	112.53±2.08	111.8±2.2
Tromexil (200)	210.13±1.33	204.8±0.58	108.96±0.97	103.38±4.05
Tolmetin (50)	209.16±0.82	228.0±1.16***	111.4±1.56	163.04±2.52***
Aspirin (100)	207.2±1.09	228.8±1.88**	111.06±0.82	145.66±3.81**
Brufen (10)	201.43±1.08	208.9±1.36*	113.0±3.3	105.56±2.29
Indomethacin (2)	207.33±1.18	223.0±2.94***	109.8±1.1	173.83±4.2***

Number of animals 6 with all the drugs.

*p < 0.05

**p < 0.01

***p < 0.001

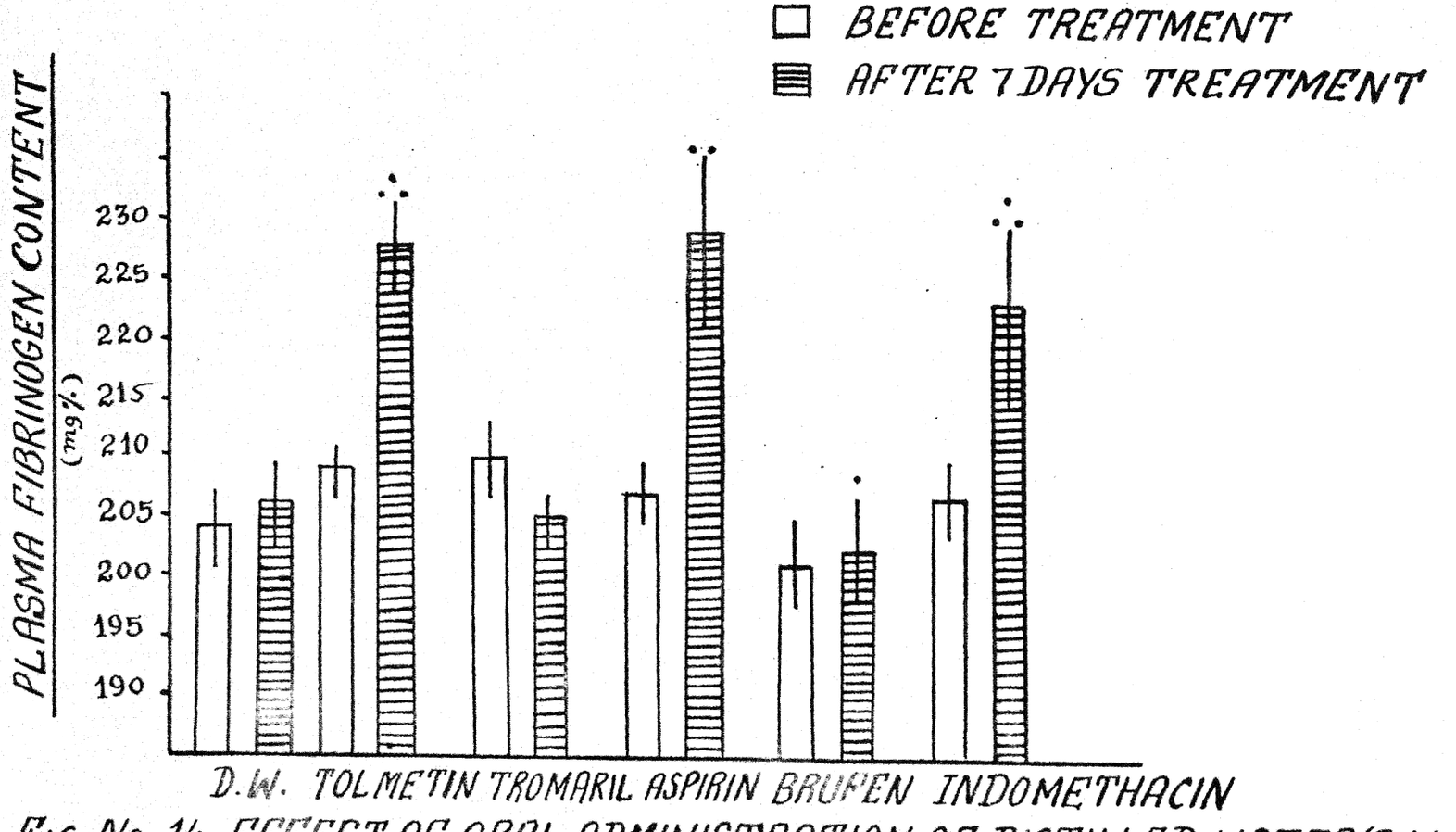


FIG. No. 14. EFFECT OF ORAL ADMINISTRATION OF DISTILLED WATER (D.W. 2 ml/kg.), TOLMETIN (50 mg/kg.), TROMARIL (200 mg/kg.), ASPIRIN (100 mg/kg.), BRUFEN (10 mg/kg.) AND INDOMETHACIN (5 mg/kg.) ON PLASMA FIBRINOGEN CONTENT IN RABBITS. WHERE *, **, *** DENOTES P VALUE < 0.05, < 0.01 and < 0.001) RESPECTIVELY.

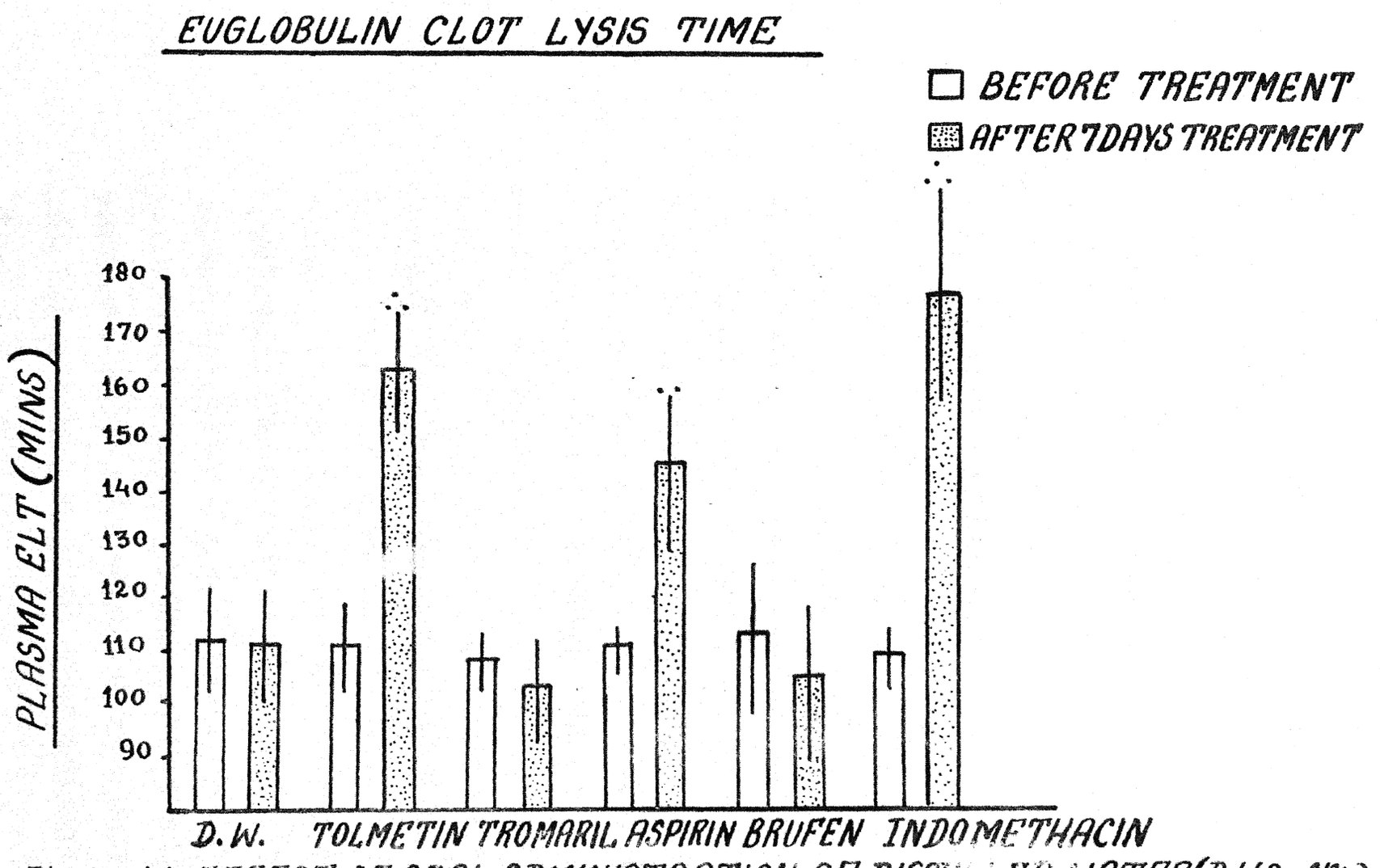


FIG. No. 15. EFFECT OF ORAL ADMINISTRATION OF DISTILLED WATER (D.W. 2ml/kg.), TOLMETIN (50 mg/kg.), TROMARIL (200 mg/kg.), BRUFEN (10 mg/kg.), ASPIRIN (100 mg/kg.), AND INDOMETHACIN (5 mg/kg.) ON EUGLOBULIN CLOT LYSIS TIME IN RABBITS. WHERE (·, ··, ∴) DENOTES P VALUES < ·05, < ·01, < ·001 RESPECTIVELY.

E.L.T. was statistically not significant (Table-6, Figure 15).

E. BIOCHEMICAL STUDIES :

The effect of these drugs was also studied on blood Sugar and serum uric acid levels in albino rabbits after oral administration.

1. Effect on Blood Sugar Level :

After 24 hours of fasting a marked variation in blood sugar level was observed in albino rabbits. The changes in blood glucose level in control group of rabbits were apparent as compared to the value at zero hour. In the subsequent studies with anti-inflammatory drugs the experiments were designed identically to control group to avoid the normal changes in blood sugar probably due to continued fasting and circandian effect. Tromaril (200 mg/kg) produced hypoglycemic effect for 2 hours showing gradual recovery at 3 hours while tromaril (250 mg/kg) induced hypoglycemia was persistant beyond 4 hours and maintained even after 7 days ($P < .01$) (Table-7, Figure 16). Indomethacin (2 mg/kg) showed peak hypoglycemic effect after two hours which was statistically significant ($P < .05$) and a gradual recovery was observed at 3 hours. Tolmetin (10 mg/kg) also produced a marked and significant hypoglycemia persisting beyond 3 hours ($P < .01$) showing recovery at 4 hours. Both aspirin (100 mg/kg) and brufen (10 mg/kg) produced a marked and highly significant hyperglycemia

TABLE - 7

Comparative effect of anti-inflammatory drugs on blood sugar level in rabbits

Group (Dose mg/kg)	Before treatment	Blood sugar mg% (Mean ± S.E.) After treatment				
		1 hour	2 hours	3 hours	4 hours	7 days
Control (2ml) (Distilled water)	110.6±2.41	102.6±2.4	102.6±2.07	100.2±2.2	98.4±3.8	109.2±1.2
Tromaril (200)	112.33±1.31	96.35±3.07	91.65±5.74	100.0±2.46	105.33±2.46	106.55±2.62
(250)	110.16±1.28	91.60±1.1 ***	94.26±2.1	94.3±0.83 ***	96.3±2.11	93.7±3.36**
Indomethacin (2)	110.26±2.42	108.06±2.83	106.31±1.5	108.84±2.17	108.55±3.30	110.4±2.1
Tolmetin (10)	110.0±2.31	105.73±1.52	80.99±6.27 ***	77.17±4.62 ***	80.5±1.64 ***	98.4±4.4
Aspirin (100)	110.5±2.87	185.70±6.90 ***	153.63±6.30 ***	113.59±3.16 ***	108.89±0.18 ***	120. ±3.5**
Brufen (10)	106.33±1.59	141.0±6.57 ***	147.13±6.50 ***	151.0±6.04 ***	155.0±6.57 ***	120.4±3.4**

Number of animals 6 with all the drugs.

* P < 0.05

** P < 0.01

*** P < 0.001

METABOLIC EFFECT

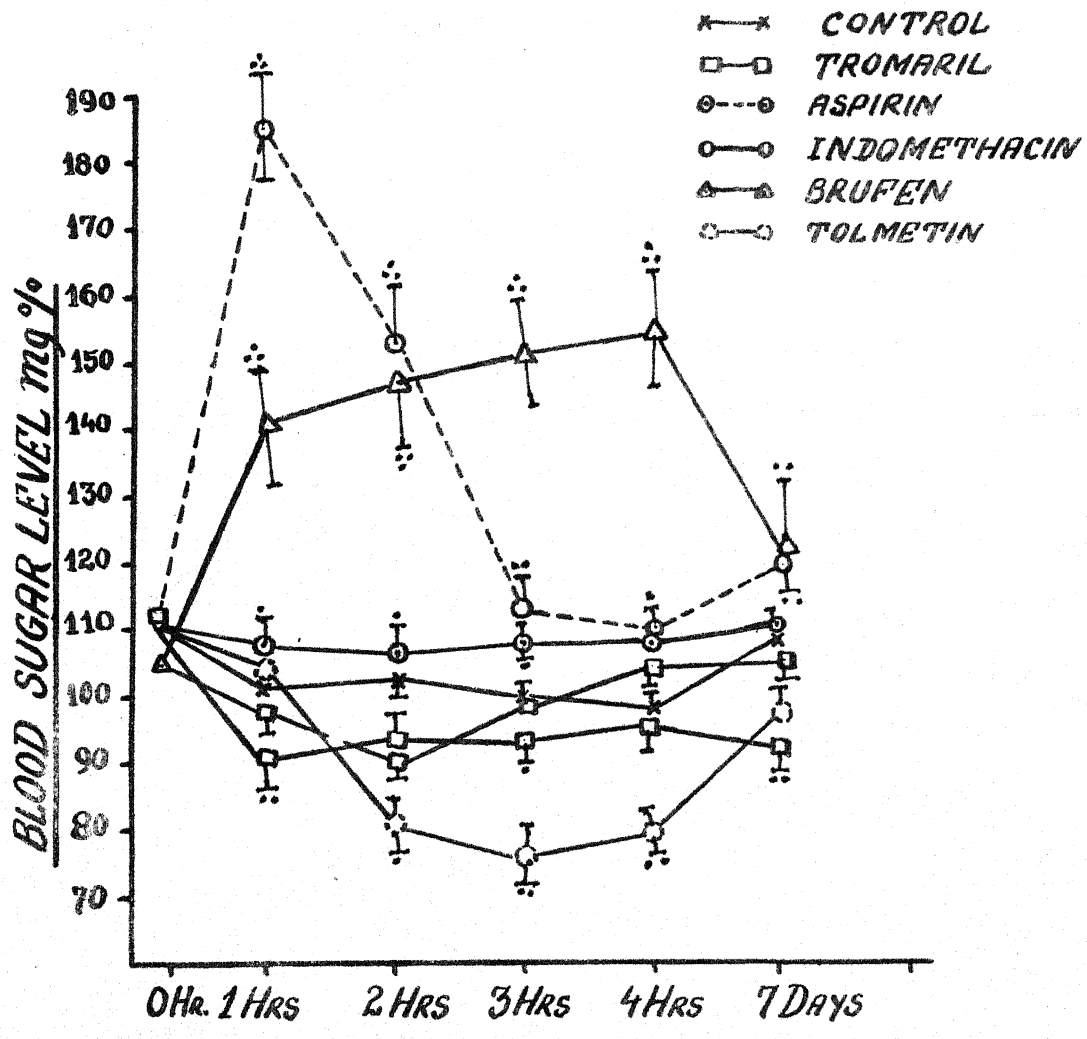


FIG. No. 16. EFFECT OF ORAL ADMINISTRATION OF DISTILLED WATER (2 mg/Kg.), TROMARIL (200 mg/Kg, 250 mg./Kg.), ASPIRIN (100 mg./Kg.), INDOMETHACIN (2 mg/Kg.), BRUFEN (10 mg./Kg.) AND TOLMETIN (10 mg./Kg.), ON BLOOD SUGAR LEVEL IN RABBITS. (*, **) DENOTES P VALUE <.05, <.01 RESPECTIVELY.

per se ($P < .001$). However, aspirin - induced hyperglycaemia was short lived upto 2 hours as compared to brufen which persistent for 4 hours (Table-7, Figure 16).

2. Effect on serum uric acid level :

In the control group of albino rabbits, the oral administration of distilled water alone showed no effect on serum uric acid level. Indomethacin (2 mg/kg) too failed to produce any significant change in the serum uric acid level upto 4 hours and even after 7 days of drug administration. However, tolmetin (10 mg/kg) produced a marked and highly significant ($P < .001$) hypouricaemic effect upto 3 hours showing recovery after 4 hours. Tromaril (200 mg/kg) lowered serum uric acid after two hours although complete recovery was not achieved even after 7 days. Aspirin (100 mg/kg) showed peak hypouricaemic effect after 3 hours which gradually recovered after 7 days and this aspirin - induced hypouricaemia was statistically highly significant ($P < .001$) after two hours. However, brufen (10 mg/kg) produced lowering of serum uric acid level which, although significant, was less marked ($P < .05$) (Table-8, Figure 17).

F. ACUTE TOXICITY STUDY :

Only 30% death occured in mice receiving tromaril in a dose of 2000 mg/kg orally. The animals showed sedation, decreased motor activity and respiratory stimulation at this dose level which disappeared within two hours and all

TABLE - 8

Comparative effect of few anti-inflammatory drugs on serum uric acid level in Rabbits

Sl. No.	Group (Dose mg/kg)	Before treatment	Serum uric acid level (in mg%) (Mean ± S.E.) After treatment				
			1 hour	2 hours	3 hours	4 hours	7 days
1.	Control (2ml) (Distilled water)	2.95±0.13	2.87±0.13	2.88±0.13	2.84±0.11	2.88±0.12	2.76±0.24
2.	Indomethacin (2)	2.90±0.18	2.90±0.03	2.77±0.05	2.82±0.05	2.78±0.04	2.74±0.04
3.	Tolmetin (10)	2.76±0.13	2.07±0.15$^{\#\#}$	1.86±0.12$^{*\#\#}$	1.63±0.13$^{*\#\#}$	2.28±0.12$^{\#\#}$	2.38±0.05*
4.	Trocmaril (200)	2.84±0.06	2.64±0.19	2.43±0.19$^{\#}$	2.28±0.17$^{\#}$	2.35±0.16	2.56±0.25*
5.	Aspirin (100)	2.76±0.14	2.56±0.13	2.25±0.06$^{\#\#}$	2.05±0.03$^{\#\#}$	2.26±0.06$^{\#}$	2.45±0.20*
6.	Brufen (10)	2.8±0.16	2.57±0.03	2.50±0.05	2.23±0.18$^{\#}$	2.53±0.05$^{\#}$	2.68±0.22*

Number of animals six with all the drugs.

*P < 0.05

**P < 0.01

***P < 0.001

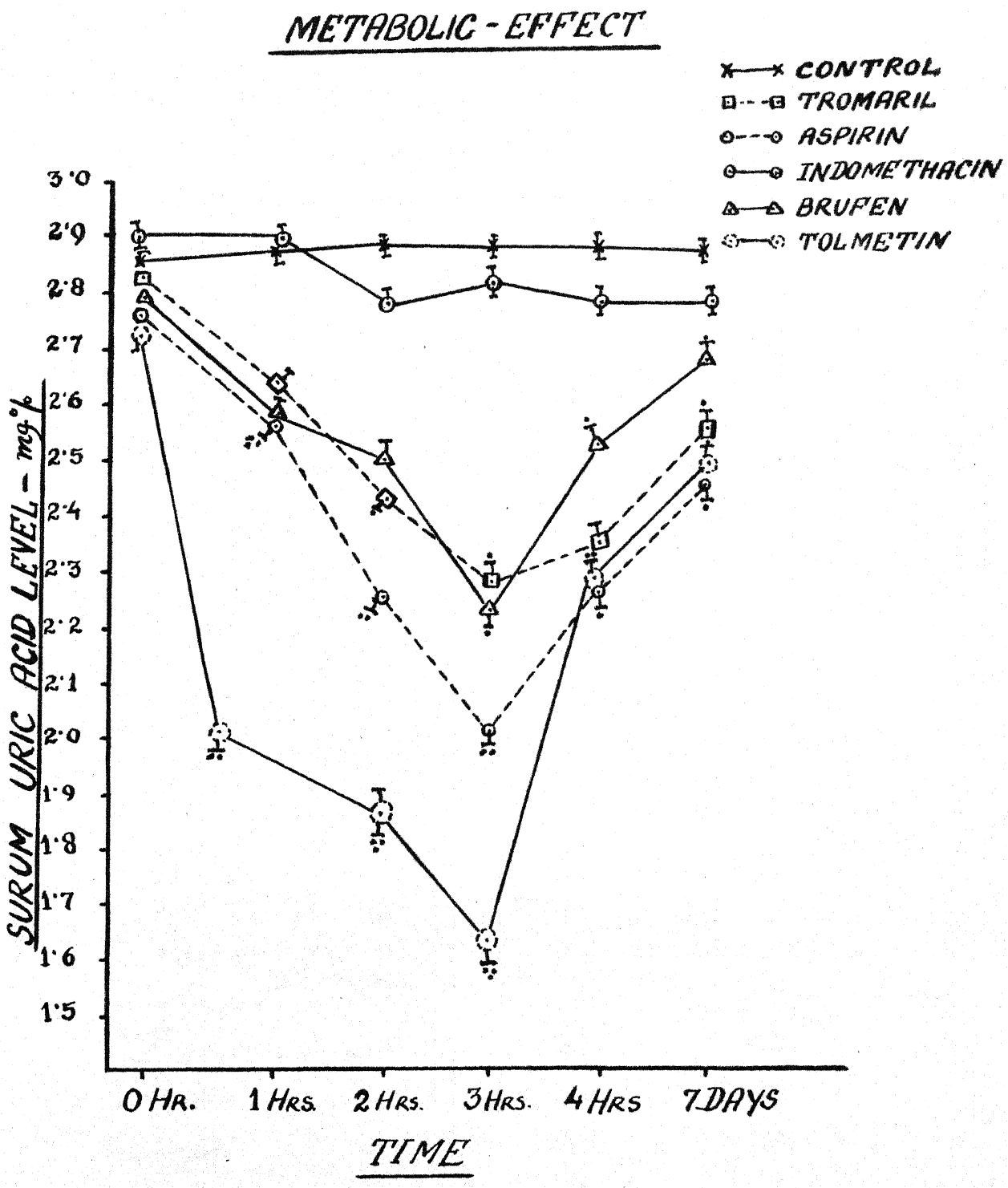


FIG. No. 17. EFFECT OF ORAL ADMINISTRATION OF DISTILLED WATER (2 mg./Kg.), TROMARIL (200 mg/Kg), ASPIRIN (100 mg/Kg.), INDOMETHACIN (2 mg./Kg.), BRUFEN (10 mg/Kg.) AND TOLMETIN (10 mg/Kg.), ON SERUM URIC ACID LEVEL IN RABBITS. (•, ••, •••), DENOTES P VALUE <.05, <.01, <.001, RESPECTIVELY.

TABLE - 9 (a)

Comparison of safety of anti-inflammatory drugs in animals(analgesic)

Drug	LD50 mg/kg	ED50 mg/kg	Therapeutic index
Tromaril	7 2000	141.3	14.15
Aspirin	1750	31.62	55.34
Tolmetin	300	52.48	5.71
Brufen	1100	17.78	61.87
Indomethacin	8.0	5.0	1.6

TABLE - 9 (b)

Comparison of safety of anti-inflammatory drugs in animals(anti-inflammatory)

Drug	LD50 (mg/kg)	ED50 (mg/kg)	Therapeutic index
Tromaril	7 2000	132.0	15.15
Aspirin	1750	98.0	17.86
Tolmetin	300	49.0	6.12
Brufen	1100	15.0	73.33
Indomethacin	8.0	5.0	1.6

surwing animals became normal. Oral LD_{50} value could not be assessed due to difficulty in administring orally a thick pasty suspension of tromaril at doses higher than 2000 mg/kg.

Aspirin 50% mortality was recorded in mice after oral administration of 1750 mg/kg of aspirin. Convulsion and increased respiration were seen in the animals.

Tolmetin (300 mg/kg) resulted in 50% mortality in mice. Increased motor activity was noted in animals after this dose.

Brufen LD_{50} value of brufen was found to be 1100 mg/kg when administered orally. During this period decreased motor activity and stimulation of respiration was observed.

Indomethacin For indomethacin LD_{50} value on oral administration in mice was calculated to be 8 mg/kg. Animals treated at this dose level manifested decreased motor activity and respiratory stimulation (Table-9).

CLINICAL STUDY :

Twenty patients of different types of arthritis (8 male and 12 female) were selected for study on various parameters like grip-strength, walking time, digital joint circumference, morning stiffness, E.S.R., Pain, fever and side effects study was undertaken in two groups of 10 patients each One group receiving aspirin served as control group and the other receiving tromaril served as the treated group. Aspirin was administered in dose of

2400 mg daily in divided doses and tromaril 1800 mg daily in divided doses.

A. GRIP STRENGTH :

Both aspirin and tromaril showed significant improvement in grip strength but aspirin produced marked and significant response after two weeks and highly significant response after four weeks of treatment ($P < .001$) (Table-10). However, tromaril showed significant response only after 4 weeks of therapy ($P < .01$).

B. WALKING TIME :

Both aspirin and tromaril gradually decreased the walking time recorded for a distance of 50 feet after two weeks of the treatment which was more marked and significant after 4 weeks of therapy (Table-11).

C. DIGIT JOINT CIRCUMFERENCE (PROXIMAL INTERPHALANGEAL CIRCUMFERENCE) :

Gradual reduction in digital joint (P.I.P.) circumference was observed during the period of active drug treatment. However, it was significant after 4 weeks of therapy in both the aspirin and tromaril - treated groups ($P < .05$) (Table-12).

D. MORNING STIFFNESS :

Both the drugs produced a gradual but significant reduction in duration of morning stiffness after one and two weeks of therapy. However, aspirin, as compared to tromaril, showed highly significant response after two weeks of therapy ($P < .001$) (Table-13).

TABLE - 10

Effect of Tromaril and Aspirin on grip-strength

Sl. No.	Treatment	No. of patients	Grip strength (mm of Hg) (Mean ± S.E.) Before	After two weeks	After four weeks
1.	Aspirin	10	31.14±5.09	59.57±9.80	74.57±5.34***
2.	Tromaril	10	26.38±5.30	36.42±5.05	52.28±4.15**

*p < .05 **p < .01 ***p < .001

TABLE - 11

Effect of Aspirin and Tromaril on walking time

Sl. No.	Treatment	No. of patients	Walking time (in seconds) (Mean ± S.E.) Before treatment	After one week	After two weeks	After four weeks
1.	Aspirin	8* (10)	28.5±4.24	24.3±4.26	21.43±3.85	16.75±2.23*
2.	Tromaril	8* (10)	33.12±4.54	29.12±4.87	26.12±4.10	21.25±3.18*

* 2 patients in each group not having involvement of the joints of the lower limbs were excluded.

*p < .05
**p < 0.01
***p < 0.001

TABLE - 12

Effect of Aspirin and Tromaril on digital joint (P.I.P.) circumference

Sl. No.	Treatment	No.of patients	Total joint circumference (in mm) (Mean ± S.E.) Before treatment	After two weeks	After four weeks
1.	Aspirin	5*	58.6±4.58	49.4±2.56	46.6±2.39*
2.	Tromaril	6*	60.16±2.35	57.83±1.99	53.0±1.99*

*Patients not having involvement of proximal interphalangial joints (P.I.P) were excluded.

*P < 0.05 **P < 0.01 ***P < 0.001

TABLE - 13

Effect of Aspirin and Tromaril on duration of morning stiffness

Sl. No.	Treatment	No.of patients	Duration of morning stiffness (in min.) (Mean ± S.E.) Before treatment	After one week	After two weeks
1.	Aspirin	10	99.37±12.69	36.68±4.09**	20.62±11.0***
2.	Tromaril	10	71.25±11.36	35.62±10.69*	22.5±7.5**

*P < 0.05

**P < 0.01

***P < 0.001

E. E.S.R. :

After 4 weeks of treatment statistically significant fall in E.S.R. was observed in aspirin treated patients while the fall in E.S.R. was insignificant in tromaril treated group (Table-16).

F. PAIN ASSESSMENT :

Aspirin treated patients reported highly significant improvement in pain after two weeks of treatment ($P < .001$). However, tromaril too showed significant improvement in pain, although less marked, as compared to aspirin ($P < .01$) during the periods of active drug treatment (Table-16).

G. FEVER :

The initial average temperature in aspirin group was 39.14°C. It came down by 1°C after 30 minutes. The fall was significant statistically ($P < 0.01$). The average maximum fall was 1.56°C after 3 hours which was maintained low as compared to initial mean temperature at statistically significant level upto 8 hours.

The initial average temperature in tromaril group was 38.85°C. It came down by 0.54°C after 30 minutes. The fall was not statistically significant ($P > 0.05$). The average maximum fall was 1.29°C after 3 hours which was statistically significant ($P < 0.05$).

At 6 hours, the temperature showed an upward trend to 38.05°C and remained almost at that level till 8 hours. The difference became insignificant from initial mean temperature.

TABLE - 14

Patients' subjective assessment of changes in pain

Sl. No.	Treatment	No. of patients	Points scored for pain (Mean ± S.E.)		
			Before treatment	After one week	After two weeks
1.	Aspirin	10	2.8±0.13	1.3±0.21**	0.4±0.16***
2.	Tremaril	10	2.5±0.16	2.1±0.23**	1.2±0.20**

*P < 0.05 **P < 0.01 ***P < 0.001

TABLE - 15

Effect of Aspirin and Tremaril on pyrexia

Sl. No.	Treatment	No. of patients	Average body temperature (in °C), hours after drug administration. (Mean ± S.E.)				
			Before treatment	½ hour	3 hours	6 hours	8 hours
1.	Aspirin	10	39.14±0.16	38.14±0.25**	37.58±0.23 ***	37.70±0.20 **	38.94±0.17 **
2.	Tremaril	10	38.85±0.23	38.611±0.28	37.56±0.25 *	38.05±0.18	38.29±0.13

*P < 0.05
**P < 0.01
***P < 0.001

TABLE - 16

Effect of Aspirin and Tromaril on E.S.R.

Sl. No.	Treatment	No. of patients	E.S.R. (in mm) (Mean ± S.E.) Before treatment	After four weeks
1.	Aspirin	10	53.60±5.32	29.82±3.77**
2.	Tromaril	10	56.70±3.57	47.60±3.88

*P < 0.05
**P < 0.01
***P < 0.001

TABLE - 17

Side effects observed during 4 weeks treatment period

Side effects	Tromaril-treated	Aspirin-treated
Nausea	3	5
Vomiting	1	2
Burning sensation in epigastrium	1	2
Melena		2

The initial average temperature in aspirin treated patients was observed to be 39.14°C. It declined to 37.58°C after 3 hours and was maintained almost at that level at 6 hours. At 8 hours, the body temperature went upto 38.94. These changes in average body temperature were statistically significant. Tromaril treated group showed an initial average body temperature to be 38.85°C which showed gradual but significant antipyretic activity after 3 hours ($P < .01$). At 6 hours the temperature showed an upward trend to 38.05°C and remained at that level upto 8 hours although not significant (Table-15).

H. SIDE EFFECTS :

During the period of active drug treatment, aspirin was found to have more potentiality for causing adverse effects as compared to tromaril. In aspirin treated patient five complained of nausea, two vomiting, two burning sensation in epigastrium while in tromaril treated only three patients complained of nausea, vomiting and one pain in epigastrium.

DISCUSSION

DISCUSSION

Struggle goes on unabated in laboratories and clinics in search of safer and potent drugs for the treatment of rheumatoid arthritis. The anti-inflammatory agents, being used clinically, possess remarkable pharmacological and biochemical activities. Non-steroidal anti-inflammatory agents with diverse chemical structure have been used in the last decade in clinical practice. However, these newer anti-arthritic drugs act as double-edged weapons because besides anti-inflammatory activity they are also prone to cause adverse effects of varying intensities. However, undesirable effects which accompany their use aroused interest in the development of more effective and safer non-steroidal anti-inflammatory drugs (NSAID). It remains debatable whether adverse effects of anti-arthritic drugs occur by the same mechanism which is responsible for their anti-inflammatory action.

Amongst the non-steroidal anti-inflammatory agents, anthranilic acid derivatives like mefenamic acid evoked interest in view of their anti-inflammatory activity. Though potent, these drugs are not devoid of undesirable effects on gastrointestinal and haematological systems. Recently, tromarit (N-3-phenylethyl anthranilic acid) was reported by Nicodia et al. (1980) as a potent anti-arthritic drug. This drug was found to possess potent analgesic, antipyretic and anti-inflammatory activities with minimal side effects. It has been described to have

an edge over the existing drugs due to wider margin of safety. However, the effects of tromaril on various haematological and biochemical parameters have not been studied in detail. Therefore, the present study was undertaken to evaluate the effects of tromaril on various biochemical and haematological parameters usually influenced by anti-inflammatory agents. Besides, comparative study was also done between tromaril and other non-steroidal anti-inflammatory drugs like aspirin, indomethacin, brufen and tolmetin for assessment of their relative analgesic, antipyretic and anti-inflammatory activities in experimental animals while clinical study was undertaken for tromaril and aspirin on various parameters. An attempt was also made to determine their safety margin for various activities.

In the present study, tromaril, aspirin, indomethacin, tolmetin and brufen produced dose-related inhibition of carrageenin-induced oedema. Tromaril elicited highly significant dose-related anti-inflammatory activity. In this study tromaril (100 mg/kg) produced 36.36% inhibition of carrageenin-induced hind paw oedema whereas same dose caused 50% inhibition in the study of Siscdia et al. (1980). Indomethacin (5 mg/kg) and tolmetin (30 mg/kg) reduced carrageenin-induced oedema by 54.54% and 36.36%, respectively which was highly significant and confirms the earlier observations (Goodman and Gilman, 1980; Sangal, 1982). While tolmetin caused inhibition of

carrageenin-induced oedema by 36.36%, 50% and 57.2% in doses of 30 mg, 50 mg, 100 mg/kg. This study suggests that comparatively the effective anti-inflammatory dose to tromaril was highest (ED_{50}= 132 mg/kg) and of indomethacin the lowest (ED_{50}= 5 mg/kg). The relative potency of anti-inflammatory agents in the present study was found to be indomethacin > brufen > tolmetin > aspirin > tromaril. However, tromaril had the highest LD50 value (> 2000 mg/kg) thus it is comparatively safer than the other anti-inflammatory agents (therapeutic index = 15.15). Bhargava et al., (1976) have also reported the order of potency of anti-inflammatory agents on carrageenin-induced oedema in following decending order : indomethacin > flufenamic acid > hydrocortisone > oxyphenbutazone > acetylsalicylic acid > amidopyrin > glycyrrhelic acid > phenacetin > sodium salicylate.

In the present study, all the anti-inflammatory agents conferred protection in animals from acetic acid-induced writhing (Table-3). While aspirin (50 mg/kg) protected 100% animals, brufen (20 mg/kg) could protect 60% animals. Tolmetin in dose of 50 mg/kg protected 40% of animals and tromaril (150 mg/kg) could only protect 60% animals. Thus it appears to be less effective analgesic as compared to the other anti-inflammatory agents. The present study showed the highest effective dose of tromaril as compared to the other anti-inflammatory agents under study (ED_{50}= 141.3 mg/kg). As regards the analgesic

potency evaluated in this study - indomethacin 7 brufen 7 aspirin -/ tolmetin 7 tromaril. As tromaril shows highest LD_{50} value it appears comparatively safer than other anti-inflammatory agents used as analgesics (therapeutic index 14.15). It has been reported that most of the anti-inflammatory drugs possess anti-pyretic acpivity (Winter et al., 1963). In our study too all the drugs studied significantly decreased the T.A.B. vaccine-induced pyrexia (Table-4). Comparatively tromaril was found to be equipotent to aspirin in antipyretic activity. It is in agreement with the observations of Sisodia et al. (1980) who reported tromaril along with aspirin, mefenamic acid and oxyphenbutazone to possess equal anti-pyretic activity in yeast-induced pyrexia in rats. Tromaril has also been observed to possess antipyretic activity in brewer's yeast induced pyrexia in rabbits (Sisodia et al., 1980). Our findings confirm the postulation that aspirin and tolmetin reduce the magnitude and duration of anti-pyretic activity (Niemegeers, 1975; Sengal, 1982).

Anderson (1965) and Green et al. (1965) drew a parallelism between anti-inflammatory and ulcerogenic activities of anti-rheumatic drugs. One of the objects of synthesis of tromaril was to introduce a better tolerated drug. Dyspepsia (Muir, 1963; O'Brien, 1968), gastrointestinal haemorrhages and perforation (Alvarez and Summerskill, 1958) of ulcers induced by the other non-steroidal anti-inflammatory agents is always

encountered in prolonged therapy. Our studies show that higher doses of tromaril (400 mg/kg) were less ulcerogenic than the lower dose of aspirin (200 mg/kg), indomethacin (4 mg/kg), tolmetin (200 mg/kg) and brufen (10 mg/kg). Aspirin, indomethacin and tolmetin showed marked increase in ulcer index in stress-induced and pyloric ligation-induced gastric ulcerations. However, tromaril and brufen slightly potentiated the ulcer index by Shay's technique and stress-induced ulcers. In view of the foregoing, tromaril appears to be less ulcerogenic and safer than the other antiinflammatory drugs.

In the present study, both aspirin and brufen produced a marked and highly significant hyperglycaemia. Aspirin has been found to elicit dual response on the carbohydrate metabolism. On one hand, it tends to lower the blood sugar level while on the other, it is known to cause hyperglycaemia, glycosuria and depletion of liver and muscle glycogen probably by releasing epinephrine consequent to activation of central sympathetic centres and partly by reducing aerobic metabolism of glucose (Goodman and Gilman, 1980). Sharma et al., (1981) reported the hyperglycaemic response of ibuprofen. It lends credence to our contention. We observed that indomethacin and tolmetin induced hypoglycaemia. Sangal (1982) has also reported the hypoglycaemic activity of indomethacin and tolmetin. This further supports our observation. Indomethacin has been reported to inhibit

the hyperglycaemia induced by angiotensin (Singh et al., 1978) and glucagon-induced hepatic glucose production (Ganguli et al., 1978). Tromaril did not effect the carbohydrate metabolism in usual doses but in higher doses it was found to cause persistent hypoglycaemia. Tromaril is known to be highly protein bound and slowly released from binding sites (Sisodia et al., 1980) so it might be responsible for sustained hypoglycaemia for prolonged period in higher doses. On effective dose basis, tromaril as compared to the other anti-inflammatory agents in this study, does not appear to affect the carbohydrate metabolism.

Anti-inflammatory drugs have been reported to possess uricosuric action (Yu and Gutman, 1959), consequently lowering the uric acid level in serum. In our study, it was observed that tromaril possesses significant hypouricaemic activity. However, indomethacin (2 mg/kg) failed to show any change in the serum uric acid level (Mankeri et al., 1980). Tolmetin (10 mg/kg) showed a marked and highly significant hypouricaemic activity. These observations are in agreement with Sangal (1982) and brufen also produced hypouricaemia. Aspirin has been reported to lower plasma urate levels (Goodman and Gilman, 1980). Thus tromaril appears comparatively less potent in reducing hypouricaemia.

The haemopoietic system is also influenced by anti-inflammatory agents as aspirin and indomethacin are

reported to possess anti-platelet action and also reduce the release of platelet bound C-serotonin (Zucker and Peterson, 1970). In our study, indomethacin (5 mg/kg), tolmetin (50 mg/kg) and aspirin were found to cause significant thrombocytopenia. Brufen decreased platelet count after 24 hours but it was more significant after 7 days. However, tromaril failed to affect the platelet count during this period. This observation is in agreement of Manikeri et al., (1980). Indomethacin was more potent in inducing thrombocytopenia as compared to aspirin, tolmetin, brufen and tromaril.

Significant reduction in clotting time by aspirin, indomethacin, brufen and tolmetin was observed in the present study. However, tromaril failed to affect the coagulation time. Similar effect has been reported by Gupta et al., (1980).

Significant increase in plasma fibrinogen content was obtained with aspirin, tolmetin, indomethacin and brufen. However, tromaril produced a decrease in the plasma fibrinogen content but it was statistically not significant. Manikeri et al., (1980) also reported minor alteration plasma fibrinogen content by aspirin and tromaril. Tolmetin, indomethacin and aspirin significantly increased euglobulin clot lysis time while decrease in E.L.T. was observed with tromaril and brufen. However, Rishi et al., (1976) observed increased fibrinolytic activity by aspirin. Done (1960) reported an in vitro

inhibition of fibrinolysin with anti-inflammatory agents. Increased plasma fibrinogen content results due to inhibition of fibrinolysis (plasma). This lends support to our observations. In the present study, plasma E.L.T. was also increased after pre treatment with aspirin, indomethacin and tolmetin. This observation further supports the presence of anti-fibrinolytic effect in anti-inflammatory agents. However, pretreatment with tromaril and brufen resulted in decreased plasma E.L.T. which needs further study to explore the mechanism involved there in.

In this study, tromaril had the highest LD_{50} value (7 2000 mg/kg p.o.) as compared to other anti-inflammatory drug. Comparative safety (based on the therapeutic index) of the anti-inflammatory agents in this study was found to be as follows in descending order brufen 7 aspirin 7 tromaril 7 tolmetin 7 indomethacin (Table-9). Thus it is evident that tromaril is comparatively a safer anti-inflammatory agent.

In the clinical study, aspirin produced significant and a higher degree of improvement in grip-strength than tromaril. An increase in grip strength has also been earlier reported with aspirin (Anseli et al, 1978), and tromaril (Sattur et al., 1980; Mathur et al., 1980). Tromaril and aspirin were equi-effective on walking time which improved appreciably and gradually as is evident in our study by a decrease in walking time. This is in agreement with the observations of Sattur et al., (1980), who clinically

examined tromarii. Significant decrease in digital joint (P.I.P.) circumference was observed with both tromarii and aspirin in patients. However, Ansell et al., (1978) reported no change in proximal interphalangeal joint measurement throughout the period of treatment, but Sattur et al.,(1980) obtained results similar to this study. In our study, aspirin was found to decrease the duration of morning stiffness significantly as compared to tromarii Sattur et al., (1980) also reported decrease in morning stiffness in tromarii-treated group and its increase in aspirin-treated patients (Ansell et al., 1978). Both the drugs were found to provide significant relief from pain subjectively, although objective assessment of relief from pain has been reported by earlier observations (Lee et al., 1973; Huskisson, 1974). Aspirin was comparatively more effective in this respect than tromarii as evidented by our study. No significant change in level of pain was reported in aspirin-treated group by Ansell et al., (1978). However, several workers observed significant and marked relief from pain with tromarii which lends support to our observation (Mathur et al., 1980; Narshing Rao, 1980; Swamy et al., 1980; Sattur et al., 1980; Rao et al., 1980). Reduction in E.S.R. was noted in both aspirin and tromarii-treated groups. However, aspirin showed more marked reduction in E.S.R. as compared to tromarii. Sattur et al., (1980) also reported fall in E.S.R. in tromarii-treated group which lends further sup-

port to our study. Although raised E.S.R. is an invariable feature of active rheumatoid arthritis, however, Hart and Huskisson, (1972) doubt its utility as an index in evaluating the response of drug in short term clinical studies. Tromaril has been reported to be equipotent with oxyphenbutazone as assessed by improvement in grip strength and reduction in swelling of joints (Rao et al., 1980). Non-steroidal anti-inflammatory drugs like phenylbutazone and paracetamol have been reported to possess antipyretic activity in symptomatic relief of acute rheumatic fever and rheumatoid arthritis (Harper and Banica, 1979). In the present study, both tromaril and aspirin showed marked reduction in elevated body temperature, which confirms the observations of Gupta et al. (1980). However, aspirin was found to possess more significant anti-pyretic activity as compared to tromaril in onset, degree and duration of pyrexia. Thus tromaril with its reasonable antipyretic action may be of advantage in rheumatic fever and rheumatoid arthritis.

Aspirin was found to show more side effects as compared to tromaril as evidenced by the pattern of side effects in our study (Table-17). Ansell et al. (1978) also reported higher incidence of adverse effects with aspirin than tolmetin. Tromaril has been found to possess minimal side effects and good tolerance and was completely devoid of any adverse effect on G.I.T., cardiovascular or haemopoetic system (Mathur et al., 1980) indicating its safety and effectiveness in rheumatoid arthritis.

CONCLUSION

CONCLUSION

In the present study, confirmation and comparison of potency of tromaril with other non-steroidal anti-inflammatory agents like aspirin, indomethacin, brufen and tolmetin was done in experimental models and between aspirin and tromaril in patients of different types of arthritis. To assess the safety of tromaril over other anti-inflammatory agents. Toxicity studies were done and incidence of side effects were also recorded.

Following conclusions can be drawn from the observations :

EXPERIMENTAL STUDIES :

1. Our study revealed that tromaril is a weak analgesic (ED_{50}=141.3mg/kg) as compared to the other anti-inflammatory agents. The relative analgesic potency was found to be in the order - Indomethacin > brufen > aspirin > tolmetin > tromaril. Indomethacin appears to be comparatively most potent but the safety margin is higher with tromaril as evidenced by LD_{50} value being (> 2000 mg/kg) and therapeutic index (14.15).
2. In the present investigation, all the drugs were found to possess significant anti-pyretic activity in T.A.B. vaccine - induced pyrexia. However, tromaril appears to be equipotent to aspirin in this test.
3. Our experiments showed that tromaril is the least potent anti-inflammatory agent as evidenced by the

relative potency of the non-steroidal anti-inflammatory agents in decending order - indomethacin > brufen > tolmetin > aspirin > tromaril and is further supported by value of anti-inflammatory ED50 highest for tromaril (132 mg/kg) and lowest for indomethacin (5 gm/kg). However, tromaril is comparatively safer than the other anti-inflammatory agents as is evidenced by LD50 value > 2000 mg/kg.

4. Our study suggests that tolmetin, aspirin, indomethacin, brufen and tromaril possess ulcerogenic activity at a higher dose. However, tromaril proved to be comparatively less ulcerogenic than the other anti-inflammatory agents. Aspirin, indomethacin and tolmetin were found to markedly increase the incidence of ulcers following pyloric ligation or stress. Comparatively tromaril and brufen slightly proved to be safer as they increase incidence of ulcers in the above test. slightly.

5. Aspirin and brufen were found to induce hyperglycemia while tolmetin and indomethacin produced hypoglycemia. However, tromaril did not effect the carbohydrate metabolism in the usual doses while in higher doses it induced hypoglycaemia. Therefore, it can be safely inferred that tromaril does not possess any intrinsic effect on carbohydrate metabolism.

6. In our study, aspirin, brufen, tolmetin and tromaril affected the uric acid metabolism as they produced

hypouricaemia, aspirin and tolmetin were most potent while tromaril and brufen were less potent. Indomethacin was devoid of any effect on the serum uric acid level. Tromaril exhibited a weaker hypouricaemic activity as compared to aspirin and tolmetin.

7. We observed thrombocytopenic reponse with indomethacin, tolmetin, aspirin and brufen. While tromaril did not affect platelet count. Comparatively indomethacin was the most potent in inducing thrombocytopenia.

8. Clotting time was decreased by aspirin, indomethacin, brufen and tolmetin. However, tromaril did not show any change in the coagulation time. On the basis of this observation, it may be concluded that tromaril can safely be used in blood coagulation disorders.

9. Our study shows significant increase in plasma fibrinogen content and E.L.T. with aspirin, tolmetin and indomethacin while brufen showed increased plasma fibrinogen content and decreased E.L.T. However, tromaril showed insignificant decrease in plasma fibrinogen content and euglobulin clot lysis time. Tromaril, therefore, appears to possess fibrinolytic activity but needs further study for confirmation.

10. In the toxicity studies, tromaril proved to be the safest drug with LD_{50} value of more than 2000 mg/kg suggesting a wider margin of safety as compared to

the other anti-inflammatory agents. Relative safety of the anti-inflammatory agents was calculated in the descending order - brufen > aspirin > tromaril > tolmetin > indomethacin.

CLINICAL STUDY :

1. In the present study, aspirin produced slightly more significant improvementin grip strength than tromaril, indicating its superiority over the other drug.
2. Our study suggests that tromaril and aspirin are equieffective in improving walking time, signifying benefical effect.
3. The presdnt study revealed that both aspirin and tromaril significantly decreased the digital joint circumference (P.I.P.).
4. We observed that aspirin markedly decreased the duration of morning stiffness as compared to tromaril.
5. In the present investigation, both the drugs afforded significant relief from subjective pain. However, aspirin was found to be more effective than tromaril.
6. Aspirin caused significant reduction in E.S.R. as compared to tromaril.
7. Both tromaril and aspirin treated patients showed anti-pyretic activity. So tromaril with its reasonable anti-pyretic action could be of advantage in

rheumatic fever and rheumatoid arthritis.

8. Aspirin treated patients showed higher incidence of side effects as compared to tromarii. It indicates better tolerance of tromarii over aspirin.

9. It may be concluded that tromarii is a safer reasonably potent and effective anti-arthritic agent. It suppresses the process of inflammation, diminishes the severity of pain, brings down elevated body temperature improves the joint function in the patients of rheumatoid arthritis.

BIBLIOGRAPHY

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1. Adam,S.S., and Cobb,R. A possible basis for anti-inflammatory activity of salicylates and other non-hormonal anti-rheumatic drugs. Nature, Lond. 1958, 181, 773-775.

2. Alvarez,A.S. and Summerskill,W.H.J. Aspirin in rheumatoid arthritis. Lancet, 1958, 1, 920-926.

3. Anderson,K.W. The gastroenterological side effects of anti-inflammatory drugs. Symposium on non-steroidal anti-inflammatory drugs. Medicine Foundation of International Congress 1965, Series No.82, Amsterdam, 245-250.

4. Ansell,B.M.; Carter,M.E.; Gumpel,J.M.; Meacock,I.; Rosenburg,J., and Steele,E. Clinical studies with tolmetin: open study and a double-blind cross-over comparison with aspirin in rheumatoid arthritis. Reprinted from Rheumatology and Rehabilitation, 1978, 17, 3, 150-154.

5. April,P.; Albert,M.; Deighton,M.N.; Tesmule,C. Comparison of Tolmetin with aspirin in the treatment of rheumatoid arthritis in word (Ed) Tolmetin : A new non-steroidal anti-inflammatory agent. Excerpta Medica Princeton, 1975.

6. Arrigony-Martelli,E. Inflammation and anti-inflammatory. New York spectrum, 1977, 343-349.

7. Armstrong,O.; Jepsen,J.B.; Keele,C.A.; and Steward, J.M. Pain producing substance in human inflammatory exudate and plasma. J. Physiol., 1957, 135,350-355.

8. Auwerters,F.; Heimegeer,C.J.E. and Lenaerts,F.M. The effect of Suprofen in rats with mycobacterium butyricum-induced arthritis. Arzneimittel, Forschung, 1975, 15-26.

9. Bain,L.S.; El-Gheharey,A.F.; Collins,R.M.; and Sargent,N.W. Tolmetin : an evaluation of a new preparation in the treatment of rheumatoid arthritis. Brit.J.Clin.Prac.,1975,29,208.

10. Best,C.H., and Taylor,N.B. Physiological basis of Medical Practice, 10th edition. The William and Wilkins Co. Baltimore, 1979, 4-83-4-84.

11. Bokemair,H.; Giessler,A.J.; and Hirschelmann,R. Prostaglandins and inflammation. Pol.J. Pharmac.Pharm.,1974,26,5.

12. Bhall,T.N., and Tangri,K.K. The time course of the carrageenin induced oedema of the paw of the rat. J.Pharm.Pharmacol.,1970,22,721.

13. Bhargava,K.P.; Gupta,M.B., and Tangri,K.K. Mechanism of ulcerogenic activity of indomethacin and oxyphenbutazone Europ J. Pharmacol., 1973, 22, 101-105.

14. Bhargava,K.P., and Gupta,M.B. Pharmacology of inf inflammation. Ind. J.Pharmacological education, 1977,42-56.

15. Bhargava,K.P.; Nath,R.; and Palit,C. Nature of histaminergic receptor concerned in capillary permeability. Brit.J.Pharmacol.,1976 (in press).

16. Bhatt,K.G.S., and Sanyal,R.K. Association of histamine and 5-hydroxytryptamine with inflammatory processes. J.Pharm.Pharmacol.,1963,15,78.

17. Bhalla,T.N.; Sinha,J.N.; Tangri,K.K. and Bhargava, K.P. Role of catecholamines in inflammation. Europ J. Pharmacol., 1970,13,90.

18. Biscourt,J.J.de- A comparative study of ibuprofen (Brufen) and indomethacin in uncomplicated arthritis. Curr.Med.Res.opin,1975,3,477-480.

19. Boyd, William. Inflammation and repair, eigth edition Published in Great Britain by Henry. kimpton, London,1974,76-129.

20. Boyle,J.A., and Buchanan,W.W. Clinical rheumatology Blackwell Scientific Publications, Springfield, 1971,111,60-68.

21. Brown,J.H. (1945). Biol.Chem.158,501,Quoted from Hawk's Physiological Chemistry, 14th edition (Oser,B.L. ed). Tata Mc Graw-Hill Publishing Co. Ltd., New Delhi, 1965, pp.1048.

22. Buckell,M. J. Clin. Path., 1958,2,403. Quoted from M.D. thesis of K.K. Saxena, Meerut University, 1976.

23. Buttle,G.A.H.; Arcy,P.F.D.; Howard,E.M. and Kellet, D.N. Plethysmometric measurement of swelling in the feet of small laboratory animals. Nature (London), 1957,179,629.

24. Carson,J.R.; McKnistry,D.N., and Wong,S.S. 5-Benzoyle-1-Methulpyrrole-2-acetic acid as anti-inflammatory agents. J.Med.Chem.1971,14,646-647.

25. Chemmape,M.K., and Sattur,P.B. Clinical trials of RM-6 in Rheumatoid arthritis. Report submitted to drug controller India.

26. Conheim,J. Lectures on General Pathology (English Translation), London, 1889.

27. Collier,H.O.J.; Dineen,L.C.; Johnson,C.A.; and Schneiler,C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Brit.J.Pharmacol.,1968,32,295-310.

28. Crunkhorn,P., and Willis,A.L. Action and interaction of prostaglandins administered intradermally in rat and in man. Br.J.Pharmac.,1969,36,216.

29. Crunkhorn,P., and Willis,A.L. Cutaneous reactions to intradermal prostaglandins. Brit.J.Pharmac., 1971 a,41,69.

30. Crunkhorn,P., and Willis,A.L. Interaction between Prostaglandin E and F given intradermally in rats. Brit.J.Pharmac.,1971 b, 41,507.

31. Dahanukar,A.; Khardini.; Sheth,U.K.; Bhiwankar,N.T.; Ramnath; Mehta,B.C.; and Katoch,D.S. Quantitative assessment of gastrointestinal blood loss with anti-inflammatory drugs. Jr.Asso.Phys.Ind., 1980,28,67-71.

32. Dass,M.; Gupta,M.B.; Gupta,G.P.; and Bhargava,K.P. Role of biogenic amines in the pathogenesis of gastic ulceration induced by intraperitoneal injection of aspirin in rats. Ind.J.Med.Res., 1978,67,677.

33. Dacie,J.V. and Lewis,S.M. Practical Haematology 5th ed. The English Language Book Society and Churchill Livingstone, Edinburgh, 1975, pp.53.

34. Deodhar,S.D.; Dick,W.C.; Hodgkinson,R.; and Buchanan,W.W. Measurement of clinical response to anti-inflammatory drug therapy in rheumatoid arthritis. Quart.Jour.Med.,1973,166,387-401.

35. Devi, Shoba; and Polasa,H. Preliminary studies on the mutagenicity of tromaril in mice. Jr.Asso. Phys.Ind.,1980,28,36-38.

36. Dhawan,B.N. and Srimal,R.C. Anti-inflammatory and some other pharmacological effects of 3, 4-trans -2, 2-dimethyl- 3 phenyl-4 (P- (β-pyrrolidinoethoxy)-phenyl-7-methoxychromen (Centch-

ment). Br.J.Pharmacol.,1973,49,64-73.

37. Di Rosa,M.; Giroud,M.P.; and Willoughby,D.A. Studies of the mediators of the acute inflammatory response induced in rats in different sites by Carrageenin and turpentine. J.Pathology,1971, 105,15.

38. Done,A.K. Salicylates intoxication significance of measurement of salicylates in blood cases of acute ingestion Paediatrics (Springfield), 1960,26,800-807.

39. Dresser,H., Pflugers Archiv fur die Gesamte Physiologie des Menshen und der tiere,1899,76,306.

40. Dubes,T.S., and Parker,J.M. A central component in the analgesic action of sodium salicylates Archs. Int.Pharmacodyn.Ther.,1971,194,117-122.

41. Ferreira,S.H., and Vane,J.R. New aspects of the mode of action of non-steroidal anti-inflammatory drugs. A Rev.Pharmac.,1974,14,57-73.

42. Flower,R.J. Drugs which inhibit prostaglandin biosynthesis. Pharmacol.Rev.,1974,26,33-67.

43. Folin,O., and Wu,H. A simplified and improved method for determination of blood sugar.J. Biol.Chem,1920,41,367-374.

44. Ganguli,S.; Sperling,M.A.; and Ronald,C.F. Inhibition of glucagon induced hepatic glucose production by indomethacin. Am.J.Physiol.1979,236(4), 358-63.

45. Ghosh,M.N. Toxicity studies fundamentals of experimental pharmacology. Pub.Scientific Book Agency, Calcutta,1971,84-88.

46. Giamsiracusa,J.E.; Donaldson,M.S.; Koonce,M.L.; Lefton,T.E.; Ruoff,G.E.; and Brooks,C.D. Ibuprofen in osteoarthritis. Cur.Med.Res.Opin., 1975,3,481-485.

47. Goodman,L.S., and Gilman,A.G. The pharmacological basis of therapeutics, 6th ed. Mac.Millan Publishing Co.,Inc.New York,1980,6,82-728.

48. Green,D.; Goodi,P.G.; and Wilson,A.B. The gastric irritant activity of some non-steroidal anti-inflammatory drugs. Fourth International Congress of Pharmacology,Basel,Switzerland,104.

49. Gupta,K.C.; Manikeri,S.; Paul,T.; Tilve,G.H.; Acharya,N., Vidya,; Mehta,J.M.; and Sheth,U.K. Effect of Rh-6, flurbiprofen and aspirin on platelet function and coagulation. Jr.Asso. Ind.,1980,28,80-86.

50. Gupta,C.; Kusham; Teresa,T.; Paul,; Bodani,P.V.; and Sheth,U.K. Antipyretic activity of Tremaril as compared to aspirin - A clinical study, Jr. Asso.Phys.Ind.,1980,28,109-111.

51. Harper,L., and Bonica,J.J. Drugs of choice 1978-79,Ed. by Walker Modal,C.V. Mosby Company,1979,205.

52. Hart,F.D., and Huskisson,E.C. Measurement in rheumatoid arthritis. Lancet, 1972,1,28-30.

53. Hart,F.D., and Clark,C.J.M. Measurement of digital swelling in rheumatoid arthritis. Lancet,1951, 1,775.

54. Healey,L.A.J. General practice, 1967,36,110,Quoted in recent advances in rheumatology, Churchill Livingstone, 1976,33.

55. Hench,P.S.; Kendall,E.C.; Slocumb,C.H.; and Polley, H.F. The effect of a hormone of the adrenal cortex (17-hydroxy-17-H-dehydrocorticosterone, compound E) and of pituitary adrenocorticotropic hormone on rheumatoid arthritis. Proc. staff Meet. Mayo.Clin.,1949,24,181.

56. Holdstock,D.J.; Mathias,A.P.; and Schachter,M. A comparative study of Kinin, Kallidin and Bradykinin,J. Pharmac.,1967,12,149.

57. Hurly,J.V.; and Spector,W.G. Endogenous factors responsible for leucocytic emigration in vivo. J.Path.Bact.,1961,82,403.

58. Huskisson,E.C. Measurement of pain. Lancet,2,1974, 1127.

59. Huskisson,E.C.; Dipp,P.A.; Scott,J.; Trapnell,J.; Balme,H.W.; Willioughby,D.A. Immunostimulant therapy with levimasole for rheumatoid arthritis. Lancet,1978,2,339-395.

60. Jaffe,I.A. Arthritis and rheumatism,8,1064. Quoted in recent advances in rheumatology (Ed.Buchanan, W.W. and Dick,W.C.) Churchill Livingstone,1976, 33.

61. Katona,G. Scand. Quoted in recent advances rheumatology (Ed. Buchanan,W.W. and Dick,W.C.) Churchill Livingstone, Edinburgh, 1976, 33,J.of rheumatol. Supple.,1973,2,101.

62. Kshirsagar,A. Nilima.; Athanikar,N.; and Sheth, U.K. Comparison of P.G. synthetase inhibitor activity of Tromarid (Rh-8) and indomethacin. Jr.Asso.Phys.Ind.,1980,28,39-41.

63. Kshirsagar, Nilima,A.; Shah,B.P.; Paul,T.; Acharya, V.N.; Sattur,P.B.; and Sheth,U.K. Effect of non-steroidal anti-inflammatory agents and Diuretics in Nephrotic syndrome patients. Jr. Asso.Phys.Ind.,1980,28,138-141.

64. Kshirsagar,A.; Nilima; Pohujani,S.; and Paul,T. Effect of food and formulation on Bioavailability of Tromaril. Jr.Asso.Phys.Ind.,1980, 28,64-66.

65. Kowalenski,K. Effect of certain adrenal steroids and cortisone on gastric ulcerogensis in fasting rat. Proc.Soc.Exp.Biol.Med.,1959,101, 147-150.

66. Kuehl,F.A. Jr.; Olem,H.G.; and Hem,E.A. Prostaglandin and prostaglandin synthetase inhibitors: action on cell function. In prostaglandin synthetase inhibitor (H.J. Robinson and J.R. Vane, editors), 1977,53-65.

67. Lee,P.; Webb,J.; Anderson,J.A.; and Buchman,W.W. A method for assessing the therapeutic potential of anti-inflammatory anti-arthritis.Br.Med. Br.,1973,2,685-688.

68. Lewis,G.P., Bradykinin - Biochemistry, pharmacology and its physiological role in controlling local blood flow. Scie.Basis of Med.Ann.Rev.,1962, 14,242.

69. Lewis,T. The blood vessels of human skin and their response. Shaw and Sons Ltd., London,1927.

70. Lokabai,N.; Prakash,J.; and Reddy Dharma,K. Clinical trial of Tromaril (Rh-8) in Episitemy. Jr.Asso. Phy.Ind.,1980,28,112-114.

71. Lokabai,N., and Reddy, Dharma. A double blind new crossover study of Tromaril and oxyphenbutazone and placebo in Epistemy.

72. Mac Farlane,R.C. and Pilling,J.R. Fibrinolysis its mechanism and significance. Blood,1948,3,1167.

73. Marchetti,M.; Vignati,P.; and Nicolis,F.B. Gastro-intestinal blood loss in patients receiving acetyl salicylic acid or diflene, a new anti-inflammatory drug - a comparative evaluation. Curr. Ther Res.,1974,16,742.

74. Mann,N.S. Drug induced acute erosive gastritis. American Journal of proctology,1977,28,23.

75. Manikeri,R.; Sushma,; Raghu,C.N.; Dadkar,V.M.; and Sheth,U.K. Effect of Tromaril on Platelet Function and Blood Coagulation.Jr.Asso.Phys.Ind., 1980,28,72-79.

76. Mathur,S.M.; Itigi,A.; and Begum,Sigra. Clinical Trial of Tromaril (Rh-8) in Arthritis. Jr.Asso. Phys.Ind.,1980,28,94-97.

77. Menkin,V. Biology inflammation, chemical mediators and cellular injury. Sci.,1956,123,527.

78. Muir,A. In salicylates an international symposium (Ed. Dixon,A., St.,J.; Martin,B.K.; Smith, M.J.H. and Wood,P.N.H.) 1963, Little brown and Company.

79. Naik,S.R., and Sheth,U.K. Inflammation process and screening methods for anti-inflammatory agents. Jr.Post graduate Med.,1976,22,5-21.

80. Naik,S.R., and Sheth,U.K. Some biochemical studies on N-B-phenylethyl anthanilic acid (Tromaril) A new non-steroidal anti-inflammatory agent. Jr.Asso.Phys.Ind.,1980,28,42-48.

81. Niemegeers,C.J.E.; Lenaerts,F.M.; and Janssen, P.A.J. The antipyretic effect of suprofen in rat with yeast induced fever. Arzneimittel, Forschung.,1975,25,1519.

82. Norcross,B.M. Journal of New drug,1965,5,259. Quoted in recent advancesin rheumatology (Ed Buchnan, W.W. and Dick,W.C., Churchill Livingstone,1965, 1,2,33.

83. O'Brian,W.M. Indomethacin, A survey of clinical trials. Clin.Pharmac. and Ther.1968,9,94.

84. Paalzow,L. An electrical method for estimation of analgesic activity in mice. Acta Pharmac.Succ. ., 1969,6,207-226.

85. Parratt,J.R. and West,G.B. Release of 5-hydroxy-tryptamine and histamine from tissue of rat. J.Physiol.,1957,137,179.

86. Peters,H.D.; Karzel,I.K.; Padberg,D.; Shonhoffer, P.S. and Dimmendhal. Influence of prostaglandin E_1 on cyclic 3, 5- A M P level and glycosamin-oglyian secretion of fibroblasts in vitro. J. Pharmac.Pharm.,1974,26,41.

87. Punjabi, Jamakti,; Krishna.; Usha,R.; Paul,J.; and Sheth,U.K. A comparative study of tromaril, oxyphenbutazone and placebo in episiotomy - a double blind non-cross over clinical study. Jr.Ass.Phys.Ind.,1980,28,115-117.

88. Raisz,L.G.; and Koolemans, Beymen,A.B. Inhibition of bone collagen synthesis by prostaglandin E_2 in organ culture. Prostaglandins, 1974,8,377.

89. Rao,G.N.; Jain,S.; Sisodia,P.; and Sattur,P.B. Bio-availability studies of Tromaril (RH-8) Jr.Assoc.Phys.Ind.,1980,28,60-63.

90. Rao,G.N.; Jain,S.; Itigi,A.; and Sogra, Begum. Double blind crossover study of Tromaril (RH-8) and oxyphenbutazone in rheumatoid arthritis.Jr.Asso.Phy.Ind.,1980,28,105-108.

91. Rao, Narshing,G. Preliminary trial of "Tromaril" in rheumatoid arthritis and arthralgia. Jr.Ass. Phys.Ind.,1980,28,90-93.

92. Rasta,B. Veena.; Krishna,R. Usha.; and Sheth,U.K. Tromaril excretion in breast milk of lactating women - A preliminary study. Jr.Asso.Phys.Ind., 1980,28,132-134.

93. Reddy,K. Dharma. Tromaril - A new non-steroidal anti-inflammatory drug in dentistry. Jr.Asso.Phys. Ind.,1980,28,124-125.

94. Reid,J.; Macdougall,A.I.; and Andrews,M.M. Aspirin and diabetes mellitus. Brit.M.J.,1957,2,1071-1078.

95. Rewald,E. B.C.G. in rheumatoid arthritis. Lancet, 1974,2,785-786.

96. Rishi,J.P.; Deyal,P.; and Bhandari,V.I. Effect of aspirin on blood fibrinolysis coagulation and their relation to bleeding tendencies. Jr.Asso. Phy.Ind.,1976,429-436.

97. Rocha e Silva,M.; Beraldo,W.T.; and Rosenfeld,G. Brandykinin, A hypotensive and smooth muscle stimulating factor released from plasma globulin by Shake venom and trypsin. Am.J.Physiol.1949, 156,261.

98. Rosner,B. Nonparametric methods in Fundamental of Biostatistics. Duxbury Pre Boston,1982,270-276.

99. Rosenstein,R.; and Borison,H.L. Action of carbon dioxide and sodium salicylates on central control of respiration in cats. J.Pharma.Exp. Ther.,1963,139,361-367.

100. Rossi,G.; Benefil,S.; Lieffroegh,F.; and Laubling, A.C.R. Biol.Paris,1956,150,2124. Quoted in "Evaluation of drug activities. Pharmacometrics. Vol.2,Laurence,D.R.; Bacharach,A.L. eds. Academic Press,1964.

101. Rowley,D.A., and Benditt,E.P. 5-Hydroxytryptamine and histamine as mediators of vascular injury produced by agents which damage mast cells in rats. J.Exp.Med.,1956,103,399.

102. Sangal, Neelam. M.D. Thesis, Bundelkhand University, 1982.

103. Sattur,G.B.; Channappa,N.K.; and Rajendra, Basavanna,D. Use of Tromaril (RH-8) in rheumatoid arthritis. Jr.Assoc.Phys.Ind.,1980,28,98-104.

104. Saxena,P.N. Role of prostaglandin in mediation of pyrogen fever. Ind.J.Med.Res.,1979-70,499-503.

105. Saxena,K.K.; Srivastava,R.; Kulshrestha,V.K.; and Prasad,D.N. A simple gravimatic method for estimation of plasma fibrinogen. Ind.J.Physiol. Pharmacol.,1979,23,137-139.

106. Sharma,V.V.; Srivastava,Y.K.; Kulshrestha,V.K. and Prasad,D.N. Interaction of anti-inflammatory agents with glybenclamide in rabbits. Ind.J. Pharmac.,1981,1362,207-210.

107. Shay,H.; Komarov,S.A.; Fels,S.S.; Meranze,D.; Gruenstein,M., and Siplet,H. A simple method for the uniform production of gastric ulceration in rat. Gastroenterology. 1945,5,43.

108. Shen,T.; and Winter,G. Chemical and biological studies on indomethacin sulindane and their analongs. In advances in drug research. New York. Academic, 1977,12,29-245.

109. Shen,T.V. Int.Symop. Non-steroidal anti-inflammatory drugs. Proc.1963,18.

110. Shrivar,D.A.; White,C.; Sandon,A.; and Rosenthale, M.E. A profile of the rat gastrointestinal toxicity of drugs used to treat inflammatory diseases. Toxical and app.Pharmacol.,1975,32,73.

111. Singh,K.N.; Mishra,V.; Mallick,; and Saxena,V.C. Modification of hyperglycemic effect of angiotensin by indomethacin.Ind.J.Physiol.Pharmacol., 1978,22,379-82.

122. Sisodia,P.; Rama Rao,G.S.; Sidhu,G.S.; Sattur,P.B.; and Hashim, Rizs. Proceedings of symposium on CNS-drug Published by the Council of Scientific and Industrial Research, New Delhi,1966,229-238.

113. Sisodia,P.; Ebenezer,E.S.; Rao,T. Ravindar; Bhatt, K.G.S.; Sheth,U.K.; Hashim,Riza.; and Sattur, P.B. Pharmacological studies of Tramaril (RH-8) A new anti-inflammatory drug. Jr.Asso.Phys.Ind. 1980,28,11-25.

114. Sisodia,P.; Indira,C.; Shankar, Rao,A.; Riaz,Hashim.; and Sattur,P.B. Studies of pharmacokinetics of Tramaril (RH-8) in rats and dogs. Jr.Asso.Phys. Ind.,1980,28,52-59.

115. Sisodia,P.; Vithal,P.V. Rao.; Seshagiri.; Rao,P.; and Sattur,P.B. Toxicological studies of Tramaril (RH-8). Jr.Asso.Phys.Ind.,1980,28, 26-30.

116. Sisodia,P.; Jain,S.; and Sattur,P.B. Teratological studies of Tramaril (RH-8) in rats and rabbits. Jr.Asso.Phys.Ind.,1980,28,30-35.

117. Spector,W.G.; and Willoughby,D.A. Anti-inflammatory drugs and inhibition of the kinin system. In the pharmacology of inflammation.Ed. by Deryck Tavernor and John. Tramnce,1968,66.

118. Spector,W.G. Substances which affect capillary permeability. Pharmac.Rev.1958,10,475.

119. Swamy,N.R.V.; Itigi,A.; Palsikar,R.; Murthy,N.; and Sisodia,A. Clinical study of Tramaril in various types of arthritis and arthralgia. Jr.Asso. Phys.Ind.,1980,28,87-89.

120. Talesnik,J.; and Sunahara,F.A. Enhancement of metabolic coronary dilation by aspirin like substances by suppression of prostaglandin feed-back control Nature. Lond.,1973,244, 351-353.

121. Tenney,S.M.; and Miller,R.M. The respiratory and circulatory action of salicylates. Am.J.Med.,1955, 19,498-508.

122. Valame,S.P.; Gupta,K.C.; Bavi,A.J.; and Sheth,U.K., Effect of tromaril on G6-PD deficient red cells in vitro study. Jr.Asso.Phys.Ind.,1980,28, 49-51.

123. Vane,J.R. Inhibition of prostalgnadin synthesis as a mechanism of action for aspirin like drugs. Nature, New Biol.1971,231,232-235.

124. Wallace,S.L.; Bernstein,D.; and Diamond,H. Diagnostic value of the colichicine therapeutic trial. J.AM.A.,1967,199,525-528.

125. Waltner,J.G. The effect of salicylates on the inner ear. Ann.Otal.Rhinol.Lar.,1955,64,617-622.

126. Walton,R.P.; and Darby,T.D. Circulation Research, 1958,6,155. Quoted from Goodman, L.S. and Gilman,A. A pharmacological basis of therapeutics. Mac.Publishing Co.,1976, 5 et ed 325.

127. Whithouse,M.W. and Haslam,J.M. Ability of some anti-inflammatory drugs to uncouple oxidative phosphorylation. Nature, Lond.,1962,196,1323.

128. Whitehouse,M.W. Some biochemical and pharmacological properties of the anti-inflammatory drug. Prog. Drug.Res.,1965,8,301-429.

129. Wilhelm,D.L. The mediation of increased vascular permeability in inflammation. Proc.Rev.1962, 14,251.

130. Wilhelmi,G., and Menasse-Goodynia,R. Gastric mucosal damage induced by non-steroidal anti-inflammatory agents in rats of different ages. Pharmacology,1972,8,321.

131. Willis,A.L. Release of histamine, Kinin and prostaglandin during carrageenin-induced inflammation in the rat. In prostaglandins, peptide and amines. (Mantegazza,P. and Horton,E.W.). Academic Press, London, 1969,31-38.

132. Willoughby,D.A.; and Spector,W.G. The lymph node permeability factor : A possible mediator of the delayed hypersensitivity reaction. Ann. N.Y. Acad.Sci.,1964,116,874.

133. Winder,C.V. Aspirin and algesimetary. Nature, Lond., 1959,184,494-497.

134. Winter,C.H.; Risley,E.L.A.; and Nuss,G.W. Anti-inflammatory and antipyretic activities of indomethacin-1-(P-chlorobenzoyl)-5-methoxy-2 methylindole-3-acetic acid.J.Pharmacol.Exp. Ther.,1963,141,369-376.

135. Winter,C.H.; Risley,E.A.; and Nuss,G.W. Carrageenin-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc.Soc. Exp.Biol.Med.,1962,111,544-547.

136. Wintrobe. Quoted from Hutchisons clinical Methods. Sixteenth edition. Sanford Richard, Mason Stuart, SMASH MICHAEL. The english Language Book Society and Bailliere Tindall,1975,151.

137. Wang,S.; Gardocki,J.F. and Pruss,T.P. Pharmacologic evaluation of tolectin (Tolmetin,MCN, 2559) and MCN-2891, two anti-inflammatory agents. J. Pharmacol Exp.Ther.1973,185,127-138.

138. Wong,W. Chemistry and pharmacology of tolmetin 1-methyl-5-p-toluoylpyrrole-2-acetic acid. In, Tolmetin - A new non-steroidal anti-inflammatory agent. ward,J.R. ed., Excerpta Medica, Amsterdam,1975,1-22.

139. Wolfe,J.A.; Plotzker,R.; Safirs,F.J.; Ross,M.; Popky,G.; and Rubin,W. Gastric, Genitis, and bleeding duodenal ulcer following mefenamic acid therapy. Arch.Intern.Med.,1976,136,923-925.

140. Yu,T.F.; and Gutman,A.B. Study of the paradoxical effects of the salicylates in low intermediate, high dose on the renal mechanism for excretion of urate in man. J.Clin.Invest.,1959,38,1298-1315.

141. Zucker,M.; and Peterson,J. Effect of acetylsalicylic acid, other non-steroidal anti-inflammatory agents and dipyridamole on human blood platelet. J.Lab.Clin.Med.,1970,76,66-75.

142. Zurier,R.B.; Hoffstein,S.; and Weissmann,G. Suppression of acute and chronic inflammation in adrenalectomized rats by pharmacological amounts of prostaglandins. Arthritis.Rheum. 1973,16,606.